REPORTS OF FINNISH ENVIRONMENT INSTITUTE 10| 2012

Proficiency Test SYKE 8/2011

Phytoplankton

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Finnish Environment Institute SYKE

The organizer of the intercomparison test:
Finnish Environment Institute SYKE, Laboratories
Hakuninmaantie 6, 00430 Helsinki
phone +358 20 610 123, fax +358 9 495 913
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ALKUSANAT

Suomen ympäristökeskus (SYKE) on toiminut ympäristöalan kansallisena vertailulaboratoriona vuodesta 2001 lähtien. Toiminta perustuu ympäristöministeriön määräykseen, mikä on annettu ympäristönsuojelulain (86/2000) nojalla. Vertailulaboratorion tarjoamista palveluista yksi tärkeimmistä on pätevyyskokeiden ja muiden vertailumittausten järjestäminen. SYKEn laboratoriot on FINAS-akkreditointipalvelun akkreditoima testauslaboratorio T003 ja kalibrointilaboratorio K054 (SFS-EN ISO/IEC 17025) sekä vertailumittausten järjestäjä Proftest SYKE PT01 (SFS-EN ISO/IEC 17043, www.finas.fi).

Tämä pätevyyskoe on toteutettu SYKEn vertailulaboratorion pätevyysalueella ja se antaa tietoa osallistujien pätevyyden lisäksi tulosten vertailukelpoisuudesta myös yleisemmällä tasolla. Pätevyyskokeen onnistumisen edellytys on järjestäjän ja osallistujien välinen luottamuksellinen yhteistyö.

Parhaat kiitokset yhteistyöstä kaikille osallistujille!

PREFACE

Finnish Environment Institute (SYKE) is appointed National Reference Laboratory in the environmental sector by the Ministry of the Environment according to section 24 of the Environment Protection Act (86/2000) since 2001. The duties of the reference laboratory service include providing proficiency tests and other interlaboratory comparisons for analytical laboratories and other producers of environmental information. SYKE laboratories has been accredited by the Finnish Accreditation service as the testing laboratory T003 and the calibration laboratory K054 (EN ISO/IEC 17025) and as the proficiency testing provider Proftest SYKE PT01 (EN ISO/IEC 17043, www.finas.fi).

This proficiency test has been carried out under the scope of the SYKE reference laboratory and it provides information about performance of the participants as well as comparability of the results at a more general level. The success of the proficiency test requires confidential co-operation between the provider and participants.

Thank you for your co-operation!

Helsingissä 30 Maaliskuuta 2012 / Helsinki 30 March 2012

Marja Luotola

Laboratorionjohtaja / Chief of Laboratory

1 INTRODUCTION

The Finnish Environment Institute (SYKE) is a national environmental reference laboratory established according to the Environmental Protection Act (2000). The reference laboratory of SYKE provides proficiency tests for analytical laboratories and other producers of environmental information. The proficiency testing service (Proftest SYKE) is part of the SYKE Laboratory Management System based on the EN ISO/IEC 17025 standard (2005). Majority of Proftest SYKE proficiency testing services conform the requirements of ISO/IEC 17043 (2010), ISO 13528 (2005), and IUPAC technical report (Thompson et al. 2006). The Proftest SYKE is accredited by the Finnish Accreditation Service as a proficiency testing provider (PT01, ISO/IEC 17043, www.finas.fi). However, organizing phytoplankton and zoobenthos proficiency tests do not at the moment belong to the accredited scope.

SYKE organises phytoplankton proficiency tests every other year. The phytoplankton proficiency test SYKE 8/2011 is the third virtual proficiency test of SYKE based on filmed and preserved material. The first virtual phytoplankton intercomparison test was carried out in March 2007 in co-operation with Finnish Institute of Marine Research (present SYKE, Marine Research Centre) and University of Turku (Vuorio et al. 2007a). The second test was carried out in November 2009 (Vuorio et al. 2010). SYKE has also earlier, in co-operation with University of Turku, organized three informal phytoplankton intercomparison tests, two of which were national and one international test. These tests were based on natural water samples and laboratory strains of cyanobacteria (Vuorio et al. 2007b).

Phytoplankton analyses are routinely done by one analyst. Therefore, SYKE organizes the phytoplankton proficiency tests at individual level. The participants received personal diploma including evaluation of their test results.

2. ORGANISATION OF THE PROFICIENCY TEST

2.1. Responsibilities

Contact person	Marko Järvinen[3], PhD, coordinator, person in charge Mirja Leivuori[1], SYKE reference laboratory contact person Katarina Björklöf[1], SYKE reference laboratory contact person
Expert panel	Marko Järvinen[3], PhD, Finnish Environment Institute (SYKE), Freshwater Centre Maija Niemelä[2], Finnish Environment Institute (SYKE), Freshwater Centre Reija Jokipii[2], Finnish Environment Institute (SYKE), Freshwater Centre Seija Hällfors[4], MSc, Finnish Environment Institute (SYKE), Marine Research Centre Kristiina Vuorio[2], PhD, Finnish Environment Institute (SYKE), Freshwater Centre
Invited experts	Liisa Lepistö, Professor, lake phytoplankton identification Guy Hällfors, Adjunct Professor, Baltic Sea phytoplankton identification
Address	[1]Finnish Environment Institute (SYKE), Laboratory Centre, Hakuninmaantie 6, FI-00430 Helsinki, Finland [2]Finnish Environment Institute (SYKE), Freshwater Centre, P.O. Box 140, FI-00251 Helsinki, Finland

[3]Finnish Environment Institute (SYKE), Jyväskylä office, Survontie 9, FI-40500, Jyväskylä, Finland
[4]Finnish Environment Institute (SYKE), Marine Research Centre, Erik Palmenin aukio 1, FI-00560 Helsinki, Finland

E-mail: mar ko.jarvinen@ymparisto.fi
kristiina.vuorio@ympar isto.fi

2.2. Invitation and participants

The target group of the proficiency test was consultants and environmental authorities who analyse phytoplankton samples from inland waters and/or the Baltic Sea, and phytoplankton analysts working in research institutes and universities.

Invitation to take part in the test was presented on the proficiency web page of SYKE (www.environment.fi/syke/proftest). In addition, personal invitations were sent to national and international phytoplankton expert laboratories and to European phytoplankton researchers and analysts using the e-mail lists of the Finnish Phytoplankton Society EU WISER project, HELCOM PEG (Helsinki Commission Plankton Expert Group), and EU Geographical Intercalibration Groups.

A total of 22 analysts from 19 organisations and five countries (Appendix 1, Table 1) participated in the phytoplankton proficiency test.

Table 1. Number of participants and organisations of the SYKE 8/2011 test.

Country	**No of participants**	**No of organisations**
Denmark	1	1
Estonia	2	2
Finland	9	8
Germany	4	2
Sweden	6	6
Total	**22**	**19**

3. TIMETABLE

Invitation to participate in the test was announced on September 2, 2011. The registration deadline was September 23, 2011. The test material was posted on October 7, 2011. Participants were requested to return by e-mail the test results by November 4, 2011. Preliminary results were posted to participants on November 18, 2011. The participants were asked to give their comments concerning the preliminary test results by December 9, 2011.

4. TEST MATERIAL

The test integrated three components of the phytoplankton analysis: 1) species identification, 2) phytoplankton counting and 3) measurements of cell dimensions.

The test material included two to three DVD discs with digital images for the phytoplankton identification and counting tests, and two 6 ml plastic tubes with preserved phytoplankton for the measurement test. An Excel spreadsheet template for reporting the test results was sent by e-mail to the participants. The Excel spreadsheet included detailed guidance for the test, both in Finnish and in English. The phytoplankton identification test material represented phytoplankton that typically occurs in freshwaters in the Northern Europe (lake phytoplankton identification) and in the Baltic Sea (Baltic Sea phytoplankton identification).

4.1. Phytoplankton identification test

The participants could take part in both the lake phytoplankton and the Baltic Sea phytoplankton identification tests or alternatively only one of the tests. Material for the phytoplankton identification tests was filmed using inverted microscopes with total magnifications of 250x, 750x and/or 1000x. The lake phytoplankton identification test consisted of 20 video-clips filmed from Lugol preserved samples and live material using both bright field and phase contrast illumination. A total of 20 taxa common in the Northern European freshwaters were to be identified (Figures 1a-c). The phytoplankton identification test taxa represented largely indicator species of eutrophy or oligotrophy. The Baltic Sea phytoplankton identification test consisted of 20 video-clips with a total of 20 identifiable taxa filmed from Lugol preserved samples using both bright field and phase contrast illumination (Figures 2a-c). The requested minimum level of identification (species, genus, order) was indicated in the Excel spreadsheet template.

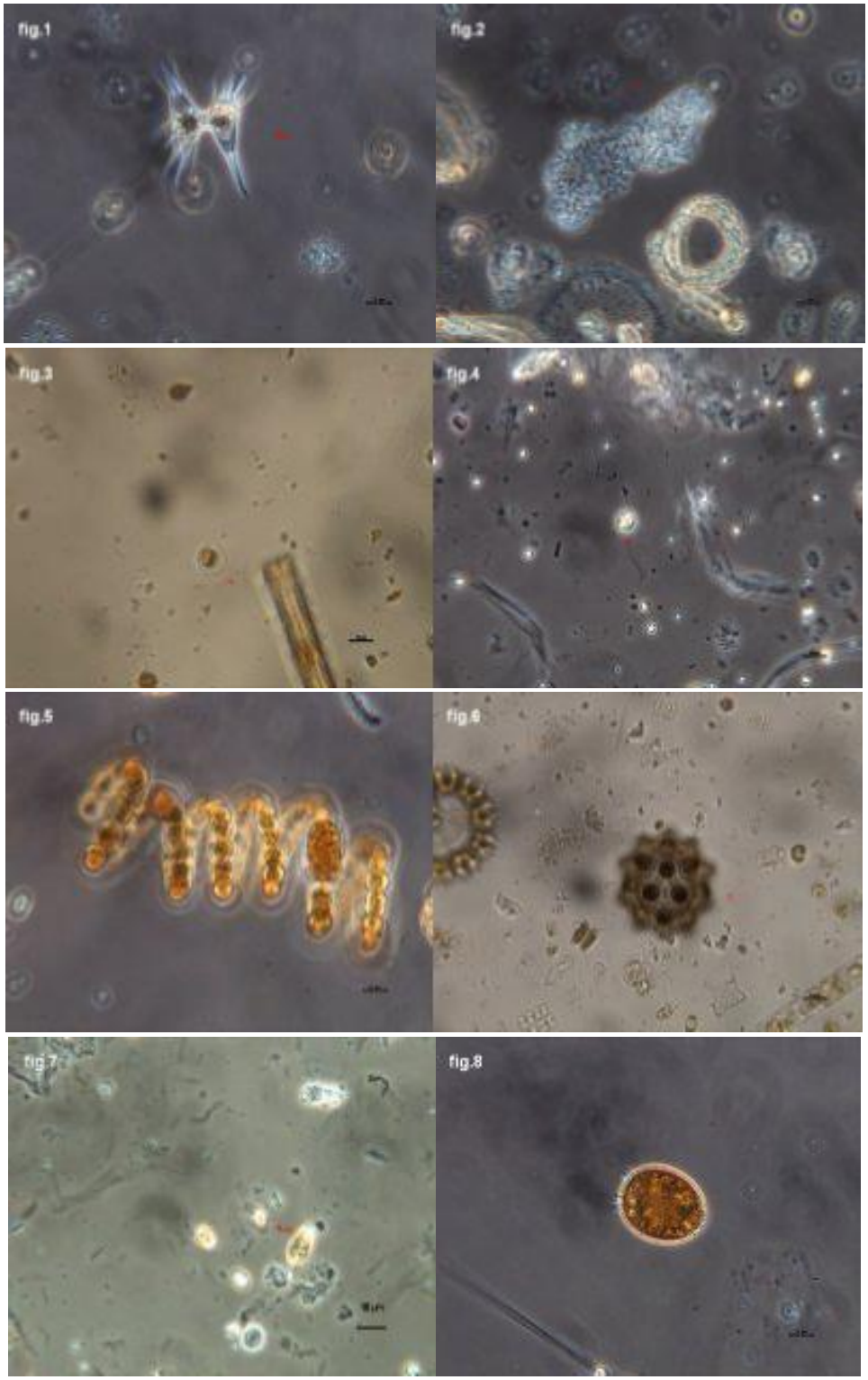

Figure 1a. Test material of the lake phytoplankton identification test comprised 20 video clips with a total of 20 taxa. Accepted identifications are given in Table 2. Resolution of the video-clips was higher than presented in these example snapshots 1-8.

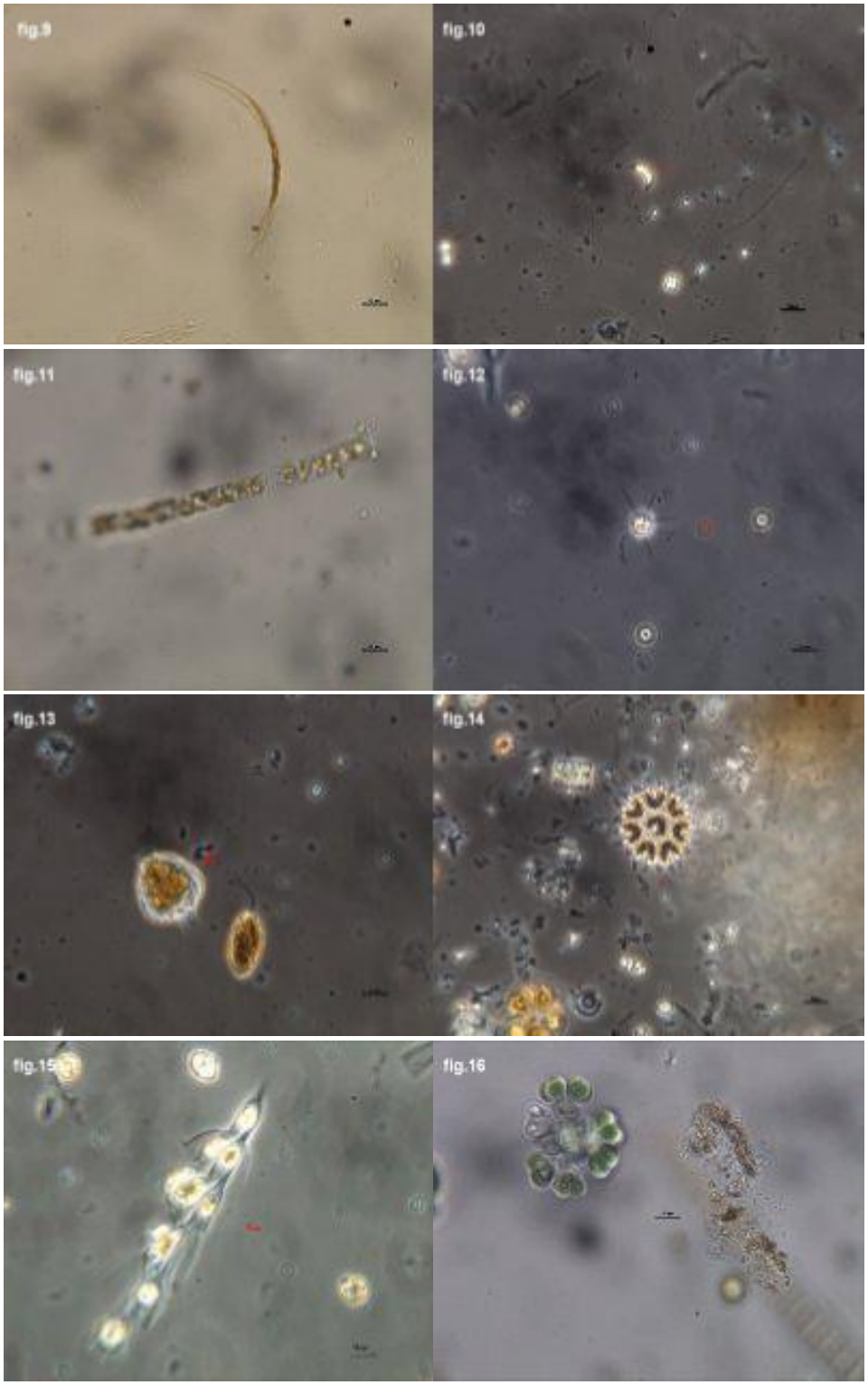

Figure 1b. Test material of the lake phytoplankton identification test comprised 20 video clips with a total of 20 taxa. Accepted identifications are given in Table 2. Resolution of the video-clips was higher than presented in these example snapshots 9-16.

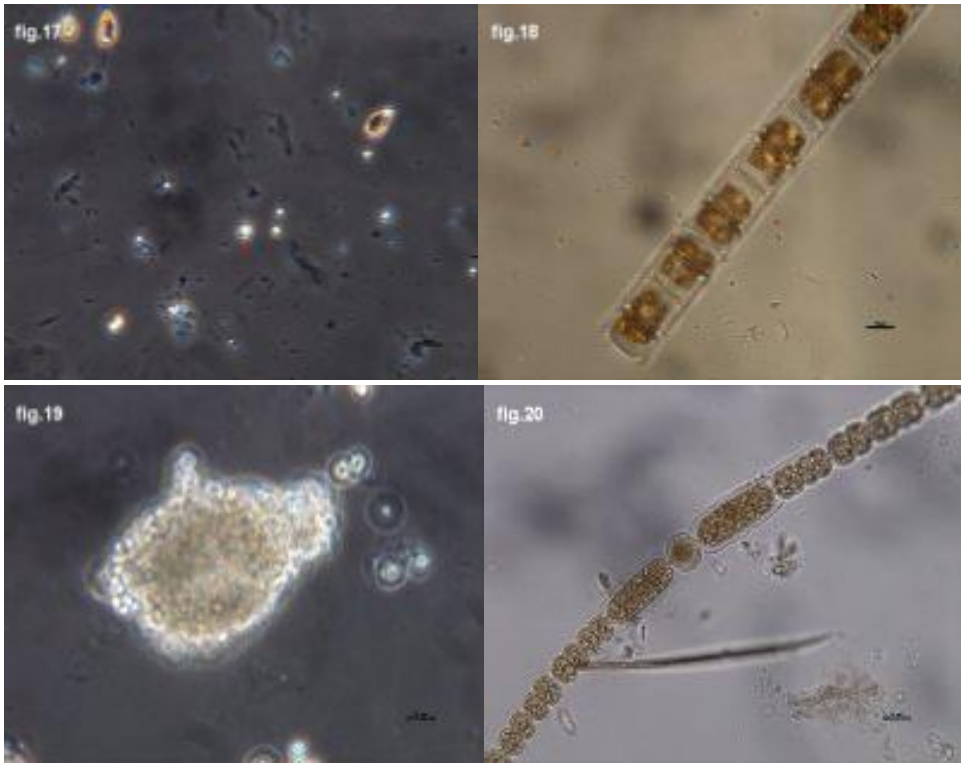

Figure 1c. Test material of the lake phytoplankton identification test comprised 20 video clips with a total of 20 taxa. Accepted identifications are given in Table 2. Resolution of the video-clips was higher than presented in these example snapshots 17-20.

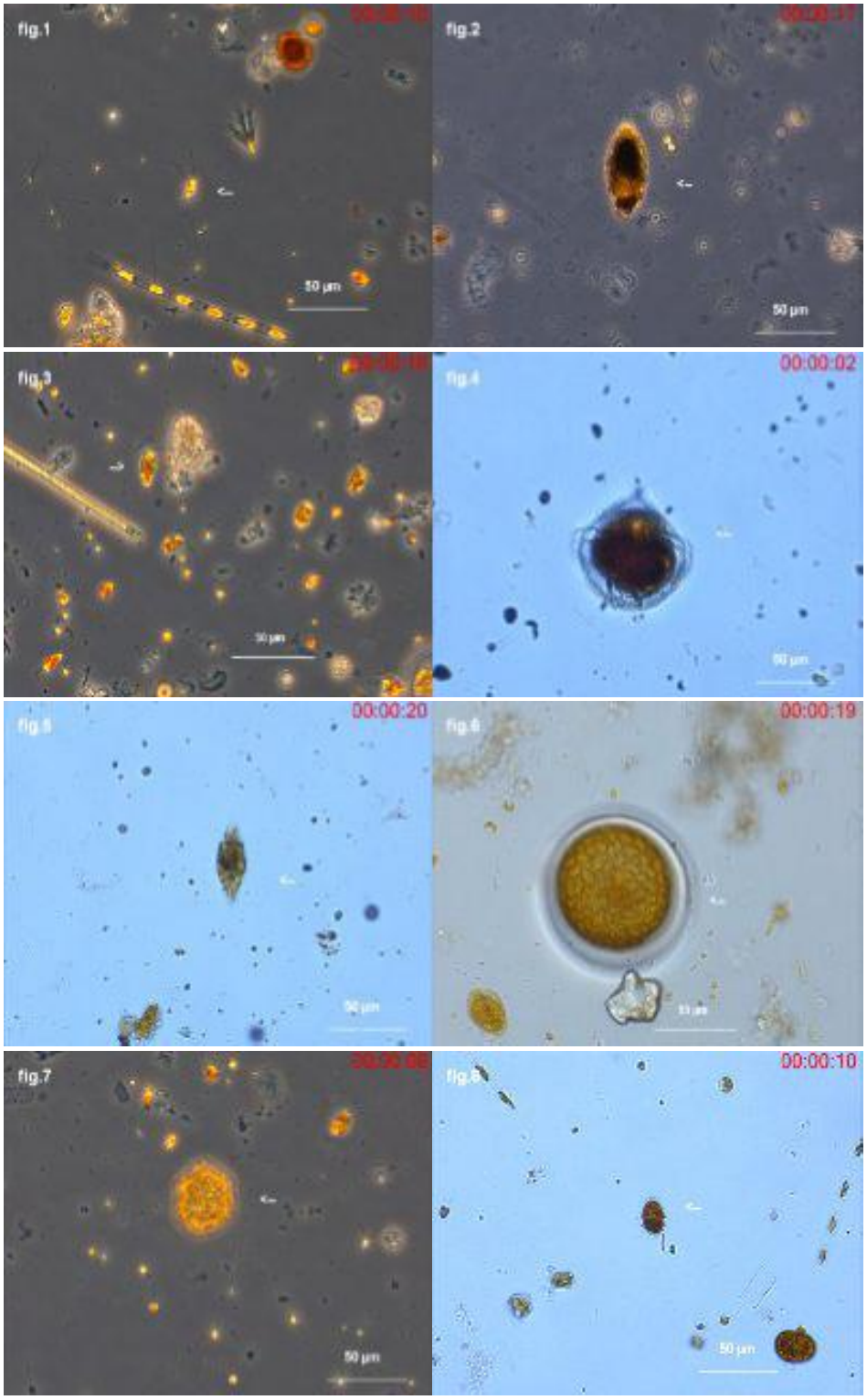

Figure 2a. Test material of the Baltic Sea phytoplankton identification test comprised 20 video clips with of total of 20 taxa. Accepted identifications are given in Table 2. Resolution of the video-clips was higher than presented in these example snapshots 1-8.

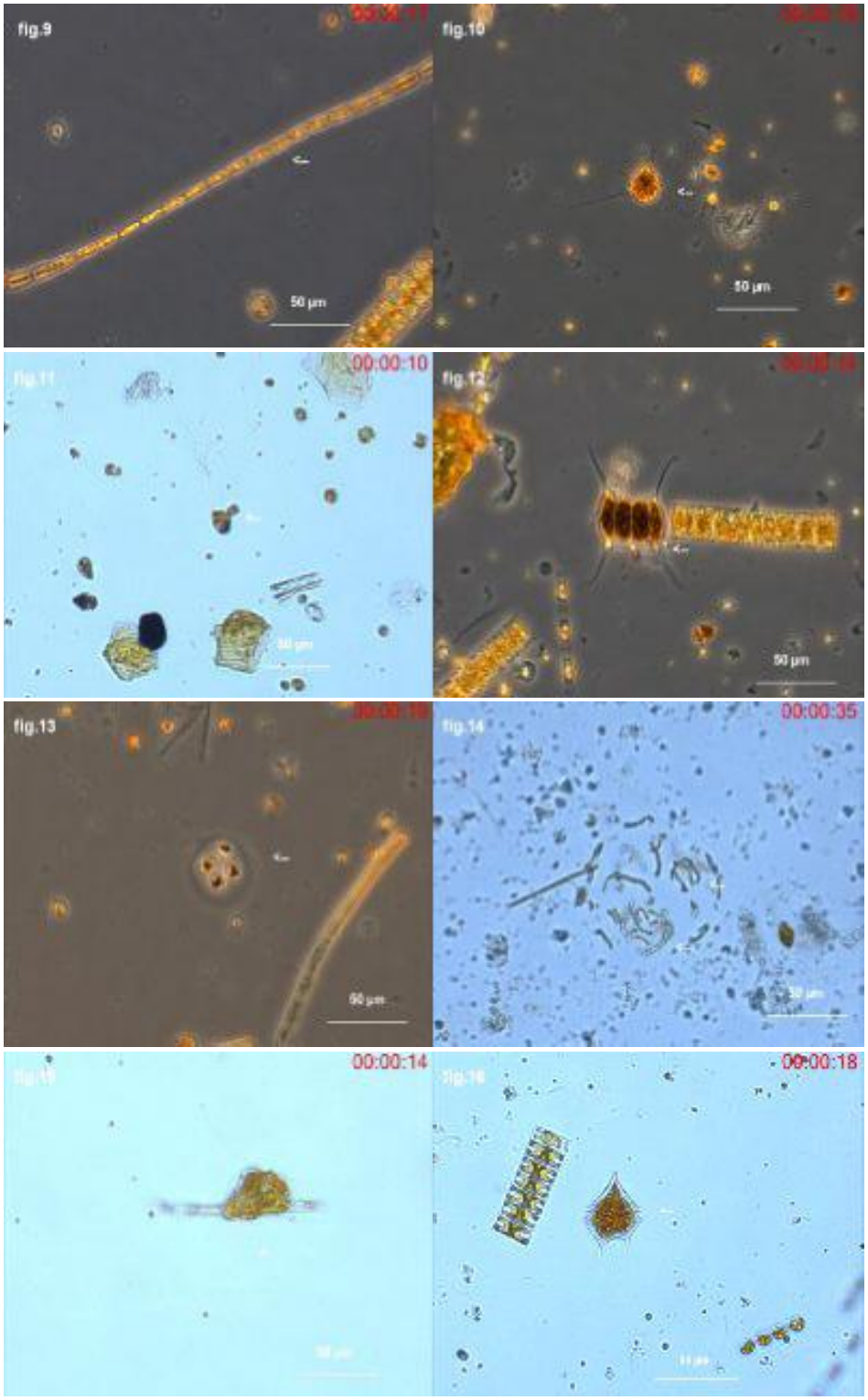

Figure 2b. Test material of the Baltic Sea phytoplankton identification test comprised 20 video clips with a total of 20 taxa. Accepted identifications are given in Table 2. Resolution of the video-clips was higher than presented in these example snapshots 9-16.

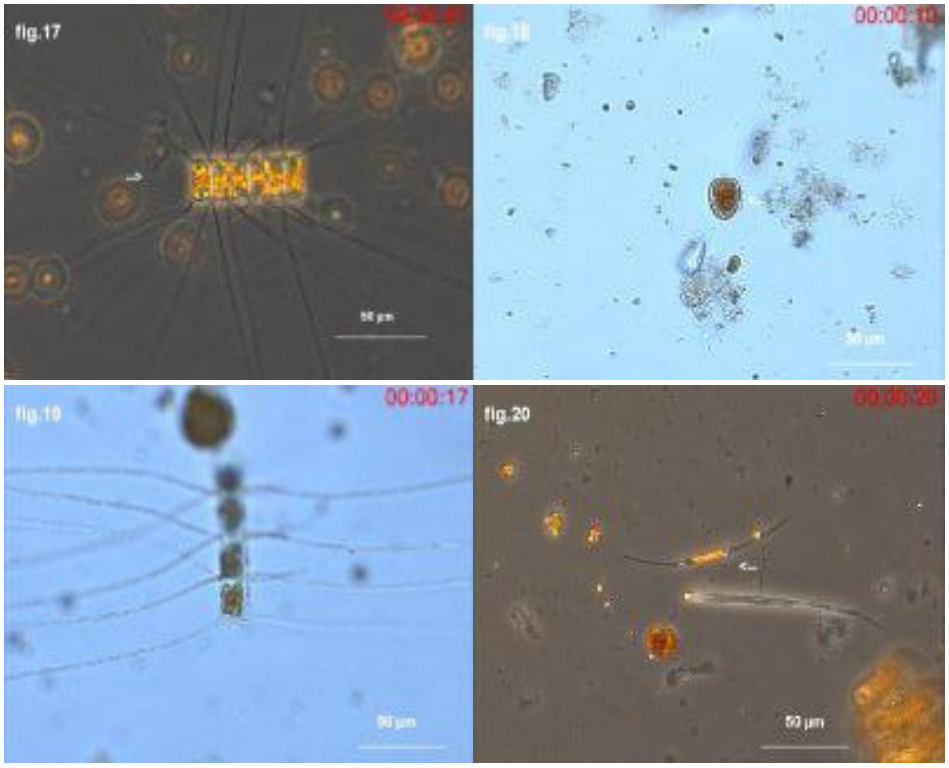

Figure 2c. Test material of the Baltic Sea phytoplankton identification test comprised 20 video clips with a total of 20 taxa. Accepted identifications are given in Table 2. Resolution of the video-clips was higher than presented in these example snapshots 17-20.

4.2. Phytoplankton counting test

For the phytoplankton counting test 25 video-clips, representing 25 fields of view in a microscope, were filmed from a composite sample that was a mixture of natural lake phytoplankton and laboratory cultures. The three test taxa consisted of a filamentous cyanobacterium *Dolichospermum* (*Anabaena*) sp. (Figure 3), a cultured pennate diatom *Diatoma tenuis* C.A. Agardh 1812 (separate cells and zigzag-like colonies) (Figure 4), and a laboratory culture of the marine centric diatom *Thalassiosira baltica* (Grunow) Ostenfeld 1901 (Figure 5). Prior to filming the composite sample was preserved with acid Lugol's solution and settled in Utermöhl settling chambers. Filming was performed using an inverted microscope with phase contrast illumination and a total magnification of 250x. The filmed material also contained other freshwater taxa originating from the lake material. The cells were advised to be counted according to EN 15204 (2006) from the counting grid that was indicated in the video-dips by red lines (Figure 6). Photographs of the requested taxa were presented in the Excel spreadsheet guidance.

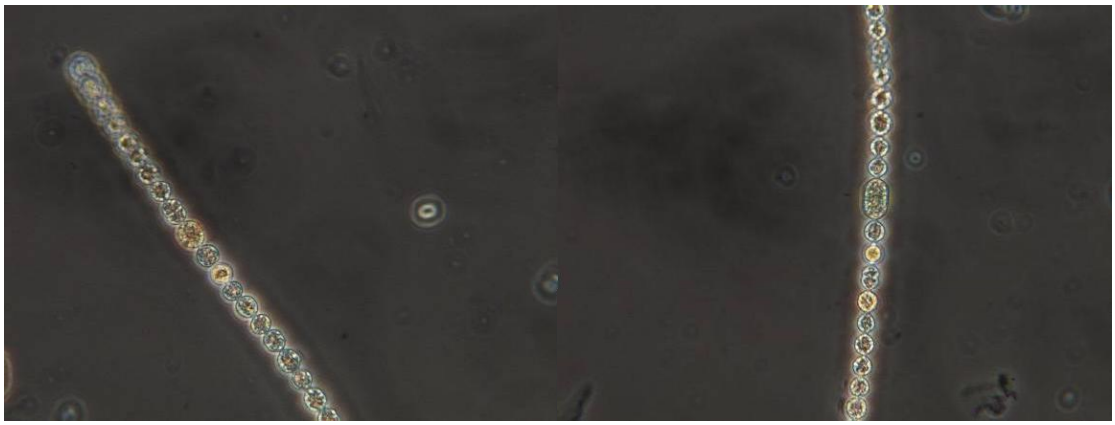

Figure 3. Cyanobacterium *Dolichospermum* (*Anabaena*) sp. represented a filamentous taxa in the counting test.

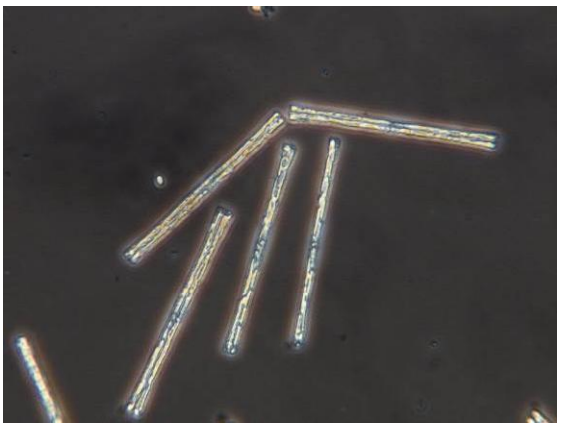

Figure 4. Diatom *Diatoma tenuis* represented a taxa forming zigzag-colonies in the counting test. *D. tenuis* was present also as separate cells.

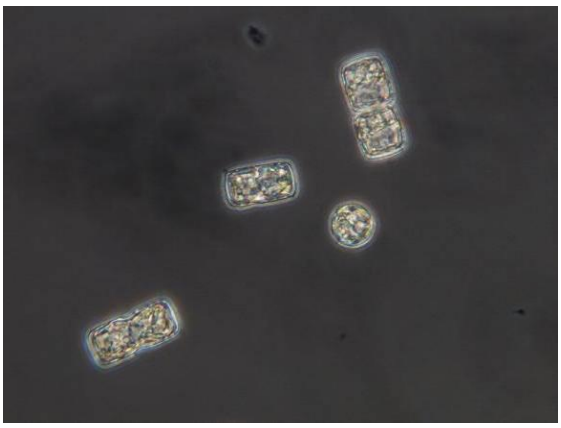

Figure 5. Diatom *Thalassiosira baltica* represented a single-celled taxa (also present as dividing cells) in the counting test.

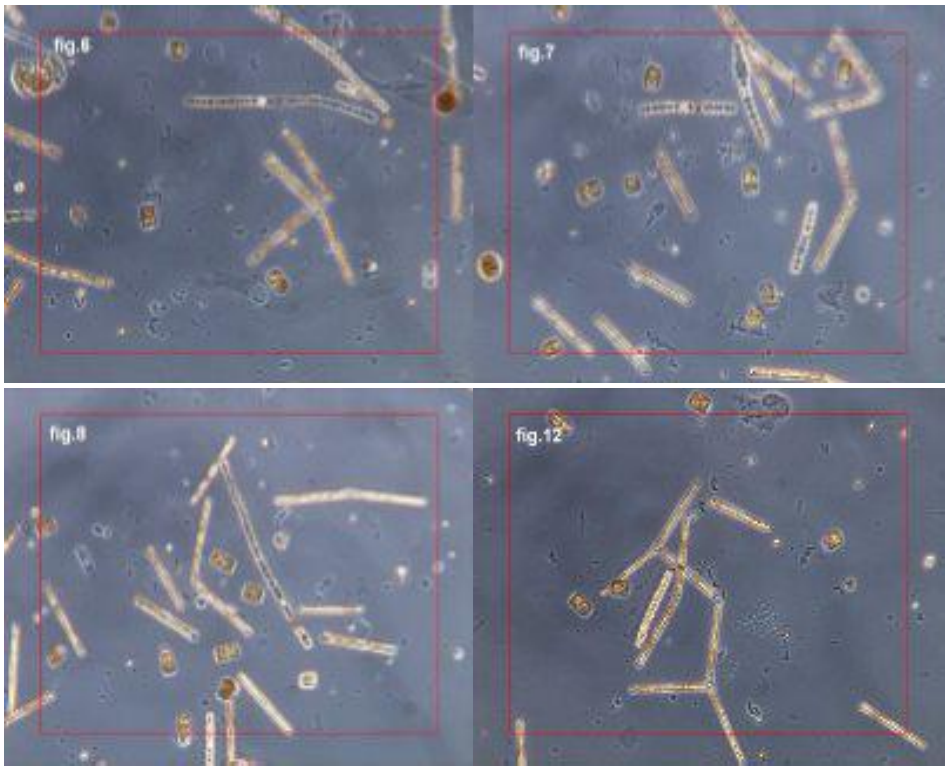

Figure 6. Example snapshots taken from the video clips filmed for the phytoplankton counting test and including the filamentous cyanobacterium *Dolichospermum* (*Anabaena*) sp., and the diatoms *Diatoma tenuis* and *Thalassiosira baltica*.

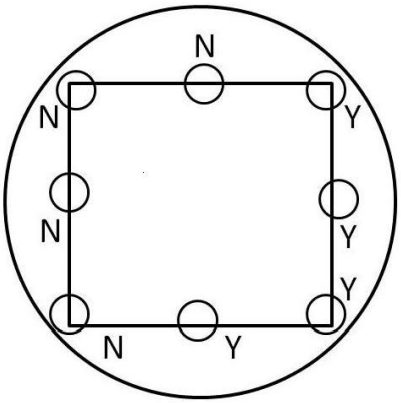

Figure 7. Recommendation of the rule for counting the cells on the edge of the counting grid as presented in the EN 15204 standard (2006) which was referred to in the SYKE 8/2011 test guidance (see also Olenina et al. 2006). In the example the objects crossing the bottom and right hand side of the grid are counted whilst those crossing both the top and left hand side of the grid are not counted. A key for the figure: Y = counted, N = not counted.

Participants were advised to perform the counting according to the guidelines presented in the EN 15204 standard (2006) (Figure 7) and report their results on the Excel spreadsheet template. The counting unit for the filamentous cyanobacteria *Dolichospermum* was a filament irrespective of its length. For the diatoms *Diatoma* and *Thalassiosira* the counting unit was a cell. Any other instructions were not given, as this part of the test also evaluated enumeration of dividing cells. Participants were also asked to describe the details of the counting method used. For the reference material of the counting test, the members of the expert panel counted the requested taxa according to the EN 15204 standard (2006) and using all possible acceptable edge combinations.

4.3. Measurement test

In the measurement test the dimensions of selected taxa were asked to be measured. For the test, a filamentous cyanobacterium *Dolichospermum* (*Anabaena*) sp. (Figure 8) from a freshwater bloom, as well as, a single-celled marine diatom (*Phaeodactylum tricornutum* Bohlin 1897) (Figure 9) and a single-celled marine dinoflagellate (*Gymnodinium corollarium* Sundström, Kremp & Daugbjerg 2009) (Figure 10), both last-mentioned from laboratory cultures, were pooled to a composite sample and preserved with acid Lugol's solution. Two replicate subsamples containing ca. 6 ml of the sample were delivered to each participant. In addition to the taxa to be measured, the sample also included other algal species.

For the filamentous cyanobacterium the cell diameter of the growing cell located in the middle of the filament was advised to be measured. A total of 20 cells were advised to be measured from different filaments, i.e. only one measurement per filament should be performed. For the diatom and the dinoflagellate, both the cell length and the width (height) of individual cells were advised to be measured. Results were reported on the Excel spreadsheet according to the guidance.

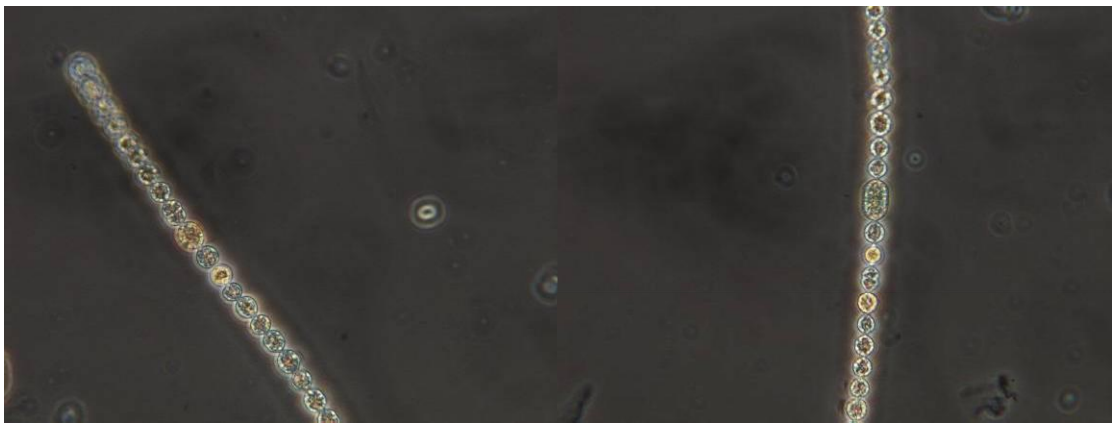

Fig 8. The cell diameter of the growing cell of the filamentous cyanobacterium *Dolichospermum* (*Anabaena*) sp., located in the middle of the filament, was advised to be measured in the measurement test.

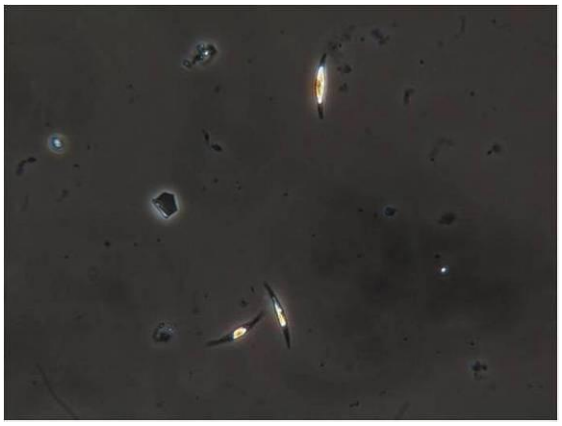

Fig 9. The frustule length and width (height) of the individual cells of the diatom (*Phaeodactylum tricornutum*) were advised to be measured in the measurement test.

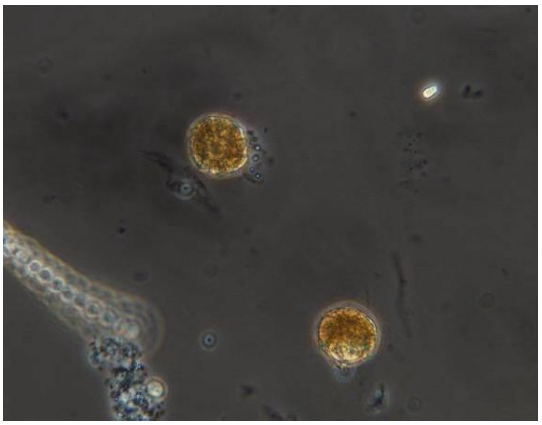

Fig 10. The cell height and the cell width of the single-celled dinoflagellate (*Gymnodinium corollarium*) were advised to be measured in the measurement test.

5. STATISTICAL ANALYSES

Statistical analyses of the counting and measurement components of the proficiency test material were carried out according to ISO 13528 (2005). Observations inconsistent with other observations, i.e. observations that were outside the 90% confidence limit, were interpreted as outliers. Thereafter, outliers were discarded on a case-by-case basis applying Hampel´s test. The robust mean values were used as assigned reference values and were evaluated applying robust statistics based on the assumption that the data are a sample from an essentially normal distribution contaminated with heavy tails and a small proportion of outliers. Therefore, normality of the results was not tested.

Uncertainty (u) of the assigned reference values was evaluated as follows: $u = 1.25*s_{rob}/\sqrt{n}$, where s_{rob} = robust standard deviation and n = number of results. The standard deviation (s_p) for the proficiency assessment was set at 10%. Criterion for the reliability of the assigned reference values was $u/s_p \leq 0.3$. This criterion was fulfilled in all statistical analysis of the test material. The criterion, $s_{rob} < 1.2*s_p$, was also fulfilled indicating that the z scores were reliable. Evaluation of performance for a single result was based on the calculation of z-scores which are deviation of the individual test results from the assigned reference values (robust mean values) compared to the target deviation (s_{target}) of 5% for the counting test. The target deviation in the measurement test was 5% for the filamentous cyanobacteria, the diatom frustule length and the dinoflagellate length and width, but 9% for the diatom frustules width (height) due to higher variation in the width of the narrow frustules. For the proficiency assessment the z-scores were considered as follows: the result was considered satisfactory if $|z| < 2$, questionable if $2 \leq |z| \leq 3$ and unsatisfactory if $|z| > 3$.

In the counting test, the results by participants, based on the counting of different edges of the counting grid, were all standardised to correspond the lower and right edge counting. The standardisation was based on the cell numbers, counted by the members of the expert panel, for all possible combinations of the diagonal edges of a counting grid according to EN 15204 (2006).

6. RESULTS

6.1 Phytoplankton identification tests

The identification results of the participants were scored 3, 2, 1 or 0 depending on the correctness of the answer (Tables 3a-b and 5a-b). Correct identification at requested identification level (species, genus, order) gave the highest score (3). Synonyms were accepted. Identification at lower level (e.g. genus level when the species level identification was requested) was awarded with 2 points. When the suggested taxon was closely related and also resembled the test taxon, 1 or 2 points were awarded depending on the estimated degree of difficulty in identification or how close relatives the respective taxa were. Each misspelling reduced the score by 0.5 points. However, Excel tends to change automatically the letter following e.g. var., did not reduce the scores. The quality target in both the lake and the Baltic Sea phytoplankton identification test was set at 75% of the maximum scores.

6.1.1 Lake phytoplankton identification test

Altogether 14 analysts took part the lake phytoplankton identification test. The requested taxa represented typical species in Northern-European freshwaters. The test taxa largely represented indicator species of either oligotrophic or eutrophic waters. The correctness of the identification of each taxon, originally carried out by the expert panel, was verified by the invited expert Professor

Liisa Lepistö. The awarded scores are presented in Table 3a-b. Only one of the taxa, the genus *Strombomonas*, was correctly identified by all participants (Figure 11). The good quality target of 75% of the maximum scores corresponded 45 points of the maximum of 60 points. Eleven participants reached the good quality target (Figure 12) and three participants failed to reach it. One of the participants received the maximum score of 60.

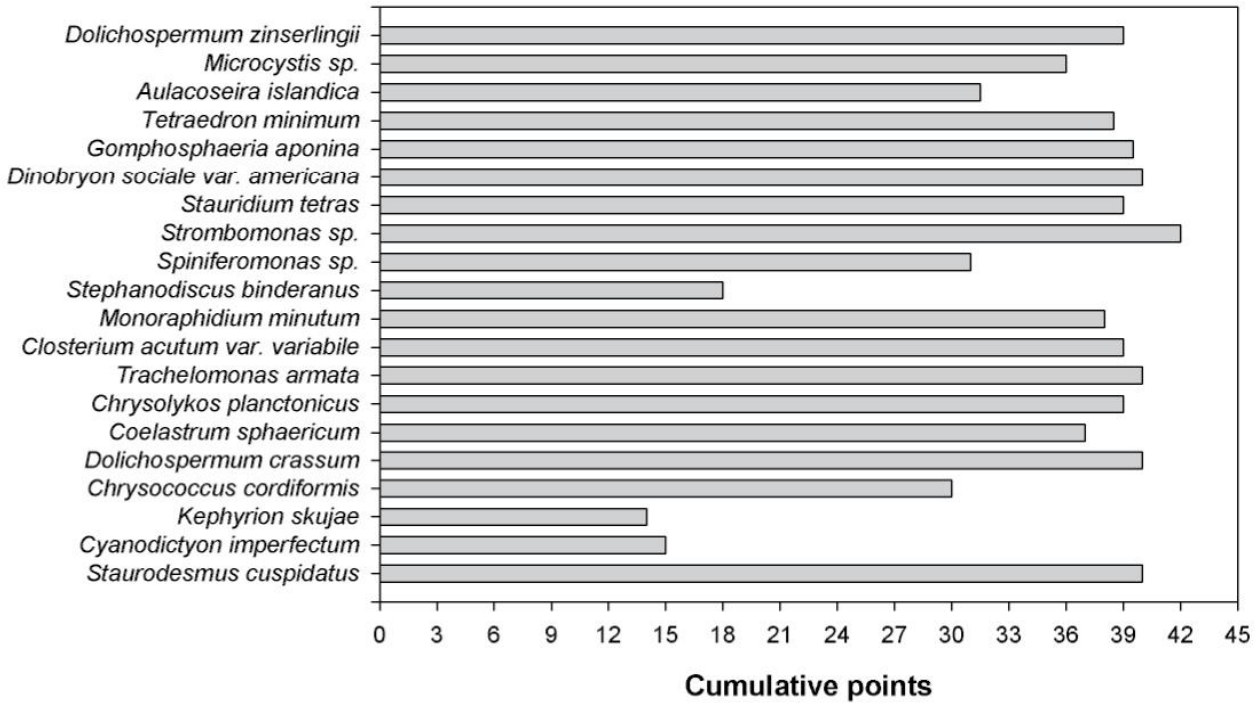

Figure 11. Cumulative points for each taxon in the lake phytoplankton identification test. Maximum score of 42 represents correct identification by all participants.

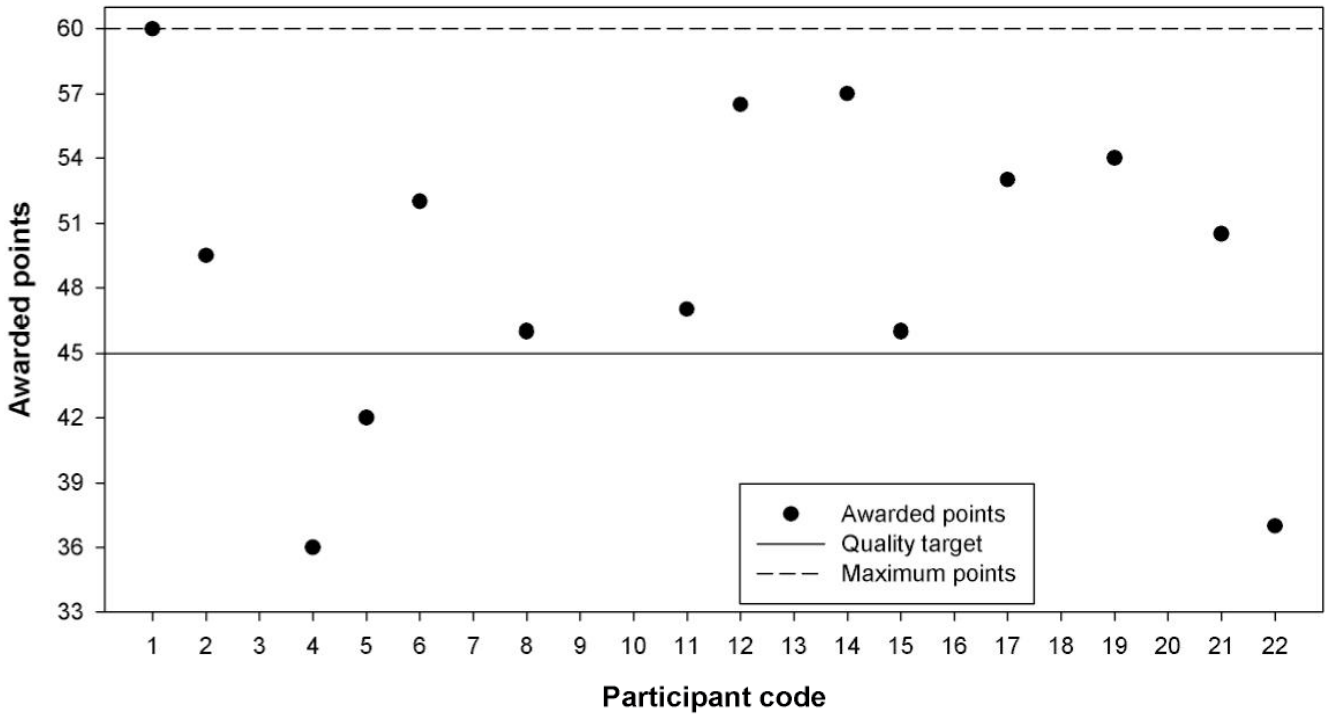

Figure 12. Results of the lake phytoplankton identification test. Satisfactory quality target was set to 45 points (≥75%) of the maximum of 60 points. Note: the Y-axis does not start at zero.

Table 2: Suggested correct identifications including the accepted synonyms in the lake phytoplankton identification test.

Video no	Accepted identification	Identification level
1	*Staurodesmus cuspidatus* (Brébisson *ex* Ralfs) Teiling 1948 [*Staurastrum cuspidatum* Brébisson *ex* Ralfs 1848]	Species
2	*Cyanodictyon imperfectum* Cronberg & Weibull 1981 [*Cyanocatena imperfecta* (Cronberg & Weibull) van Joosten 2006]	Species
3	*Kephyrion skujae* Ettl	Species
4	*Chrysococcus cordiformis* Naumann 1919	Species
5	*Dolichospermum crassum* (Lemmermann) P.Wacklin, L. Hoffmann & J. Komárek 2009 [*Anabaena spiroides* f. *crassa* (Lemmerman) Elenkin] [*Anabaena spiroides* var. *crassa* Lemmermann 1898] [*Anabaena crassa* (Lemmermann) Komárková-Legnerová & Cronberg 1992]	Species
6	*Coelastrum sphaericum* Nägeli 1849 [*Coelastrum cubicum* Nägeli 1849]	Species
7	*Chrysolykos planctonicus* Mack 1951	Species
8	*Trachelomonas armata* (Ehrenberg) Stein 1878 [*Chaetotyphla armata* Ehrenberg 1838]	Species
9	*Closterium acutum* var. *variabile* Brébisson *in* Ralfs 1848	Species
10	*Monoraphidium minutum* (Nägeli) Komárková-Legnerová 1969 [*Raphidium minutum* Nägeli 1849] [*Selenastrum minutum* (Nägeli) Collins 1907]	Species
11	*Stephanodiscus binderanus* (Kützing) W. Krieger 1927 [*Melosira binderana* Kützing 1844] [*Melosira oestrupi* Cleve-Euler 1910] [*Stephanodiscus binderanus* var. *östrupi* (Cleve-Euler) Cleve-Euler 1951]	Species
12	*Spiniferomonas* sp. Takahashi 1973	Genus
13	*Strombomonas* sp. Deflandre 1930	Genus
14	*Stauridium tetras* (Ehrenberg) E.Hegewald 2005 [*Pediastrum tetras* (Ehrenberg) Ralfs 1844] [*Micrasterias tetras* Ehrenberg 1838]	Species
15	*Dinobryon sociale* var. *americana* (Brunthaller) Bachmann 1911 [*Dinobryon americana* Brunthaller]	Species
16	*Gomphosphaeria aponina* Kützing 1836	Species
17	*Tetraedron minimum* (A. Braun) Hansgirg 1888 [*Polyedrium minimum* A. Braun 1855] [*Tetraëdron quadratum* (Reinsch) Hansgirg 1889]	Species
18	*Aulacoseira islandica* (O. Müller) Simonsen 1979 [*Melosira islandica* O. Müller 1906]	Species
19	*Microcystis* sp. Kützing *ex* Lemmermann 1907 *nom. cons.*	Genus
20	*Dolichospermum zinserlingii* (Kosinskaja) Wacklin, Hoffman & Komárek 2009] [*Anabaena zinserlingii* Kosinskaja 1929] [*Anabaena solitaria* f. *zinserlingii* (Kosinskaja) Elenkin 1938]	Species

Table 3a: Identification results suggested by the participants for each taxon (video-clips 1-11) and the corresponding awarded scores in the lake phytoplankton identification test.

Video no	Taxon	No	Points
1	*Staurodesmus cuspidatus*	7	3
	Staurodesmus cuspidatus v. *curvatus*	1	3
	Staurodesmus cuspidatus var. *divergens*	1	3
	Staurodesmus cuspitatus	1	2.5
	Staurodesmus mamillatus	2	3
	Staurodesmus triangularis	1	2
	Staurodesmus triangularis var *limneticus*	1	2
2	*Aphanocapsa delicatissima*	1	0
	Aphanocapsa holsatica	6	0
	Aphanocapsa incerta	1	0
	Cyanocatena imperfecta	1	3
	Cyanodictyon imperfectum	4	3
	Microcystis reinboldii	1	0
3	*Bicosoeca planctonica*	3	0
	Chlorella sp.	1	0
	Chrysococcus	1	0
	Chrysococcus rufescens	1	0
	Kephyrion cupuliforme	1	1
	Kephyrion skujae	4	3
	Phacotus lenticularis	1	0
	Phacotus lenticularis var *sphaerica*	1	0
	Pseudokephyrion poculum	1	1
4	*Chrysochromulina parva*	2	0
	Chrysococcus cordiformis	10	3
	Nephroselmis sp	1	0
	Trachelomonas volvocina	1	0
5	*Anabaena crassa*	7	3
	Anabaena flos-aquae	1	1
	Anabaena spiroides	5	3
	Dolichospermum crassum	1	3
6	*Coelastrum cambricum*	1	2
	Coelastrum pseudomicroporum	1	1
	Coelastrum pulchrum	2	2
	Coelastrum sphaericum	10	3
7	*Chrysolycos planctonicus*	1	2.5
	Chrysolykos planctonicus	12	3
	Kircheriella obesa	1	0
8	*Trachelomonas armata*	12	3
	Trachelomonas denisii	1	2
	Trachelomonas kelloggii	1	2
9	*Closterium acutum*	2	3
	Closterium acutum var *variabile*	1	3
	Closterium acutum var. *Variabile*	10	3
	Monoraphidium mirabile	1	0
10	*Keratococcus braunii*	1	1
	Monoraphidium minutum	12	3
	Raphidocelis subcapitata	1	1
11	*Aulacoseira islandica*	2	0
	Aulacoseira varians	1	0
	Fragilaria construens	2	0
	Hyalotheca mucosa	1	0
	Stephanodiscus binderanus	6	3
	Tabellaria binalis	2	0

Table 3b: Identification results suggested by the participants for each taxon (video-clips 12-20) and the corresponding awarded scores in the lake phytoplankton identification test.

Video no	Taxon	No	Points
12	*Chrysosphaerella* sp	1	1
	Golenkinia	1	0
	Golenkinia paucispina	1	0
	Golenkinia radiata	1	0
	Spiniferomonas	7	3
	Spiniferomonas sp.	3	3
13	*Strombomonas*	8	3
	Strombomonas sp	2	3
	Strombomonas sp.	4	3
14	*Pediastrum biradiatum*	3	2
	Pediastrum tetras	11	3
15	*Dinobryon divergens*	1	1
	Dinobryon sociale	5	3
	Dinobryon sociale var *americana*	1	3
	Dinobryon sociale var. *americana*	2	3
	Dinobryon sociale var. *Americanum*	5	3
16	*Gomphospaeria aponina*	1	2.5
	Gomphosphaeria aponina	11	3
	Gomphosphaeria natans	2	2
17	*Teilingia excavate*	1	0
	Tetraedron minimum	7	3
	Tetraëdron minimum	5	3
	Tetraëdron minutum	1	2.5
18	*Aulacoseira islandica*	9	3
	Aulacoseira italica	1	1
	Aulacoseira subarctica	1	1
	Aulacosseira islandica	1	2.5
	Melosira varians	2	0
19	*Gonyostomum sp.*	1	0
	Microcystis	7	3
	Microcystis aeruginosa	1	1
	Microcystis flos-aquae	1	2
	Microcystis sp	2	3
	Microcystis sp.	2	3
20	*Anabaena cylindrica*	1	1
	Anabaena solitaria	3	3
	Anabaena zinserlingii	8	3
	Anabaena zinzerlingii	1	2.5
	Dolichopspermum zinsenlingii	1	2.5

6.1.2. Baltic Sea phytoplankton identification test

Altogether 15 analysts took part the Baltic Sea identification test. The requested taxa represented typical species in the northern Baltic Sea ranging from common to relatively uncommon in occurrence. The correctness of the identification of each taxon, originally carried out by the expert panel, was verified by the invited expert Adjunct Professor Guy Hällfors (Table 4). Awarded scores are presented in Table 5a-b.

The identification level of the taxon no 3 (*Eutreptiella* sp.) should have been genus level, but it was erroneously reported in the Excel spreadsheet to be species level. Therefore, in addition to *Eutreptiella* sp., the species level identification of *Eutreptiella gymnastica* was awarded with three points.

Two given taxa (*Oocystis* sp. and *Cylindrotheca closterium* (Ehrenberg) Reimann & J. Lewin 1964) were identified correctly by all participants (Figure 13). The dinoflagellate *Heterocapsa arctica* ssp. *frigida* appeared most difficult to be correctly identified. None of the participants received the maximum score of 60 and five of the participants failed to reach the good quality target (Figure 14).

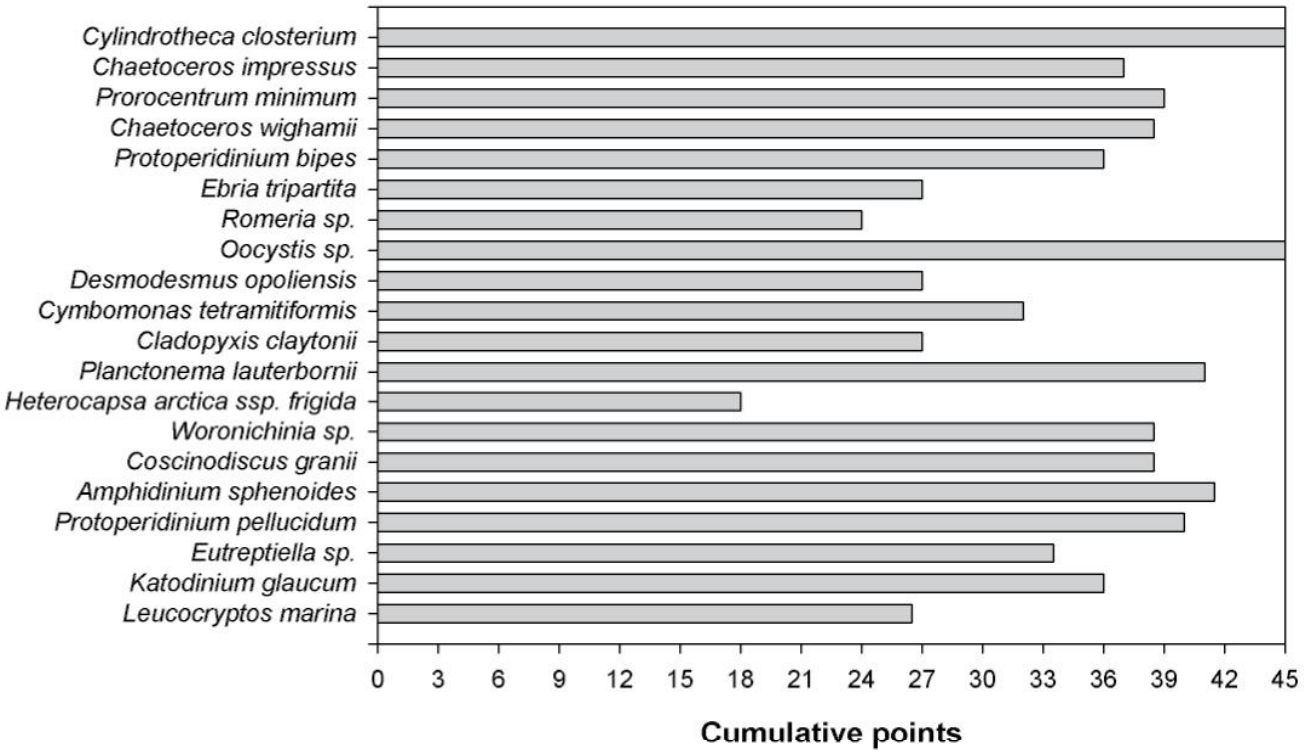

Figure 13. The cumulative points for each taxon in the Baltic Sea phytoplankton identification test. Maximum score of 45 represents a correct identification by all participants.

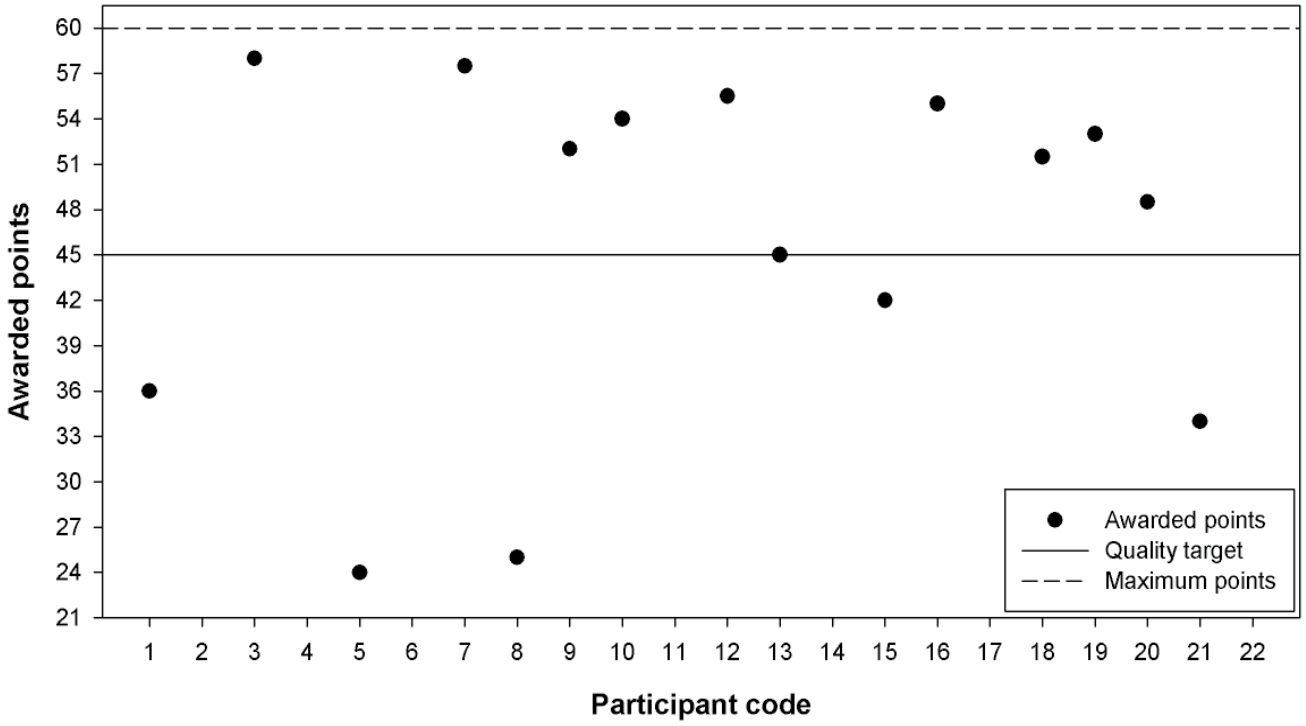

Figure 14. The results of the Baltic Sea phytoplankton identification test. Satisfactory quality target was set to 45 points (≥75%) of the maximum of 60 points. Note: the Y-axis does not start at zero.

Table 4: Suggested correct identifications for the Baltic Sea phytoplankton identification test and the synonyms as presented in the Checklist of Baltic Sea Phytoplankton Species (Hällfors 2004).

Video no	Accepted identification	Identification level
1	*Leucocryptos marina* (Braarud) Butcher 1967 [*Bodo marina* Braarud 1935] [*Chilomonas marina* Halldal 1953]	Species
2	*Katodinium glaucum* (Lebour) Loeblich III 1965 [*Spirodinium glaucum* Lebour 1917] [*Gyrodinium glaucum* (Lebour) Kofoid & Swezy 1921]	Species
3	*Eutreptiella* sp. da Cunha 1913	Genus
4	*Protoperidinium pellucidum* Bergh 1881 [*Peridinium pellucidum* (Bergh) Schütt 1895]	Species
5	*Amphidinium sphenoides* Wulff 1916 [*Gymnodinium filum* Lebour 1917]	Species
6	*Coscinodiscus granii* Gough 1905	Species
7	*Woronichinia* sp. Elenkin 1933	Genus
8	*Heterocapsa arctica* ssp *frigida* Rintala & G. Hällfors 2010	Species
9	*Planktonema lauterbornii* Schmidle 1903 [*Binuclearia lauterbornii* (Schmidle) Proschkina-Lavrenko 1966]	Species
10	*Cladopyxis claytonii* R.W. Holmes 1956 [*Micracanthodinium claytonii* (R.W. Holmes) Dodge 1982]	Species
11	*Cymbomonas tetramitiformis* Schiller 1913	Species
12	*Desmodesmus opoliensis* (P. Richter) Hegewald 2000 [*Scenedesmus opoliensis* P. Richter 1895]	Species
13	*Oocystis* sp. A. Braun 1855	Genus
14	*Romeria* sp. Koczwara *ex* Geitler 1932	Genus
15	*Ebria tripartita* (Schumann) Lemmermann 1900 (?1901) [*Dictyocha tripartita* Schumann 1867] [*Dictyocha fornix* Möbius 1887] [*Ebria fornix* (Möbius) Borgert 1891]	Species
16	*Protoperidinium bipes* (Paulsen) Balech 1974 [*Glenodinium bipes* Paulsen 1904] [*Peridinium minusculum* Pavillard 1905] [*Minuscula bipes* (Paulsen) Lebour 1925]	Species
17	*Chaetoceros wighamii* Brightwell [*Chaetoceros bottnicus* P.T. Cleve *in* Aurivillius 1896] [*Chaetoceros perpusillus* P.T. Cleve 1897?] [*Chaetoceros fallax* Proschkina-Lavrenko 1955]	Species
18	*Prorocentrum minimum* (Pavillard) Schiller 1933 [*Exuviaella minima* Pavillard 1916] [*Exuviella apora* sensu Lebour 1925 *p.p.*] [*Prorocentrum triangulatum* Martin 1929] [*Exuviaella mariae-lebouriae* Parke & Ballantine 1957] [*Prorocentrum cordiformis* Bursa 1959] [*Prorocentrum mariae-lebouriae* (Parke & Ballantine) Loeblich III]	Species
19	*Chaetoceros impressus* K.G. Jensen & Moestrup 1998 [*Chaetoceros eibenii sensu* Wołoszyńska 1935] [*Chaetoceros eibenii* f. *solitaria* Wołoszyńska 1935 *p.p.*]	Species
20	*Cylindrotheca closterium* (Ehrenberg) Reimann & J. Lewin 1964 [*Ceratoneis closterium* Ehrenberg 1841] [*Nitzschia closterium* (Ehrenberg) W. Smith 1853] [*Nitzschia curvirostris* v. *closterium* (Ehrenberg) Van Heurck 1881]	Species

Table 5a: Identification results suggested by the participants for each taxon (video-clips 1-10) and the corresponding scores in the Baltic Sea phytoplankton identification test.

Video no	Taxon	No of answers	Points
1	*Chroomonas acuta*	1	0
	Leucocryptos marina	8	3
	Leucocryptus marina	1	2.5
	Rhodomonas lacustris	1	0
	Teleaulax amphioxeia	4	0
2	*Gymnodinium vestificii*	1	0
	Gyrodinium fissum	1	0
	Gyrodinium spirale	1	0
	Katodinium glaucum	12	3
3	*Chroomonas baltica*	1	0
	Cryptomonas baltica	1	0
	Eutreppsiella gymnastica	1	2.5
	Eutreptia lanowii	1	0
	Eutreptiella cornubiense	1	1
	Eutreptiella gymnastica	9	3
	Eutreptiella sp.	1	3
4	*Gonyaulax spinifera*	1	0
	Protoperidinium granii	1	1
	Protoperidinium pellucidum	13	3
5	*Amphidinium sphenoides*	13	3
	Ampidinium sphenoides	1	2.5
	Keratococcus braunii	1	0
6	*Actinocyclus octonarius*	2	0
	Coscinidiscus granii	1	2.5
	Coscinodiscus granii	12	3
7	*Snowella*	1	0
	Snowella sp.	1	0
	Woronichinia	9	3
	Woronichinia sp	3	2.5
	Woronochinia	1	2.5
8	*Gymnodinium fuscum*	1	0
	Gymnodinium lantzschii	1	0
	Gymnodinium sp.	1	0
	Heterocapsa arctica ssp frigida	1	3
	Heterocapsa arctica ssp. frigida	2	3
	Heterocapsa arctica subsp. frigida	2	3
	Heterocapsa niei	1	1
	Heterocapsa rotundata	2	1
	Heterocapsa triquetra	2	0
	Peridinium pusillum	1	0
	Peridinium umbonatum	1	0
9	*Oscillatoria limnetica*	1	0
	Planctomena lauterbornii	1	2.5
	Planctonema lauterbornii	4	3
	Planktonema lauterborni	1	2.5
	Planktonema lauterbornii	8	3
10	*Cladopyxis claytonia*	6	3
	Heterocapsa sp.	1	0
	Micracanthodinium claytonii	3	3
	Scrippsiella trochoidea	5	0

Table 5b: Identification results suggested by the participants for each taxon (video-clips 11-20) and the corresponding scores in the Baltic Sea phytoplankton identification test.

Video no	Taxon	No of answers	Points
11	*Chrysochromulina birgeri*	1	0
	Chrysochromulina polylepis	1	0
	Cymbomonas teramitiformis	1	2.5
	Cymbomonas tertramitiformis	1	2.5
	Cymbomonas tetramitiformis	9	3
	Ochromonas sp.	1	0
	Pyraminomas sp.	1	0
12	*Desmodesmus armatus*	1	1
	Desmodesmus communis	6	1
	Desmodesmus maximus	1	1
	Desmodesmus opoliensis	1	3
	Scenedesmus opoliensis	5	3
	Scenedesmus quadricauda	1	1
13	*Oocystis*	11	3
	Oocystis sp.	4	3
14	*Aphanotheceae* sp.	1	0
	Cyanonephron	1	0
	Phormidium	1	0
	Pseudanabaena	1	0
	Pseudanabaena sp.	1	0
	Rhabdoderma sp.	2	0
	Romeria	8	3
15	.	1	0
	Ceratium divaricatum	1	0
	Ceratium sp.	1	0
	Chaetoceros danicus	1	0
	Ebria tripartita	9	3
	Protoceratium reticulatium	1	0
	Pseudochattonella farcimen	1	0
16	*Peridinium granii*	1	0
	Protoperidinium bipes	11	3
	Protoperidinium granii	3	1
17	*Chaetoceros constrictus*	1	1
	Chaetoceros holsaticus	2	1
	Chaetoceros wighami	1	2.5
	Chaetoceros wighamii	11	3
18	*Prorocentrum cordatum*	1	3
	Prorocentrum minimum	12	3
	Prymnesium parvum	1	0
	Pyramichlamys dissecta	1	0
19	*Chaetoceros ceratosporus*	2	1
	Chaetoceros danicus	1	1
	Chaetoceros impressus	11	3
	Chaetoceros wighamii	1	1
20	*Cylindrotheca closterium*	12	3
	Nitzschia closterium	3	3

6.2. Phytoplankton counting test

All 22 participants took part the counting test. Most participants carried out the counting test according to the EN 15204 (2006) as requested in the test guidance. Altogether 13 of the participants counted objects on the lower and right hand side edges, as presented in the standard example on page 14 (see Figure 7 and Tables 6-8). Other acceptable combinations were used by 6 participants. However, two participants were not aware of a proper counting procedure or did not follow the standard. One of the participants did not report the method used.

In all, 21 participants performed all components of the counting test satisfactorily ($|z\ score| < 2$). One participant failed to perform the cell count of the centric diatoms *Thalassiosira* unsatisfactorily ($|z\ score| > 3$; Tables 7-9, Figs 15-17).

Many cells of the cultured centric diatom *Thalassiosira* were undergoing cell division. No separate instructions on how to enumerate taxa were given, nor does the standard EN 15204 (2006) instruct enumeration of dividing cells. In the test evaluation, dividing cells were expected to be counted as individual cells, regardless of the stage of division (see e.g. Salas 2010).

Table 6. Total number of filaments of the cyanobacterium *Dolichospermum* (*Anabaena*) sp. in 25 video clips of the counting test. The results are presented separately for different methods for counting edges of the counting grid.

Method	N	Mean ± SD	Min	Max
Lower + right	13	47 ± 1.1	45	49
Upper + right	2	53 ± 0.0	53	53
Lower + left	2	45 ± 0.5	44	45
Upper + left	2	50 ± 0.0	50	50
Not according to EN 15204	2	47 ± 1.0	46	48
Not given	1	49		

Table 7. Total cell numbers of the pennate diatom *Diatoma tenuis* in 25 video clips of the counting test. The results are presented separately for different methods for counting the edges of the counting grid.

Method	N	Mean ± SD	Min	Max
Lower + right	13	154 ± 2.1	148	157
Upper + right	2	143 ± 0.5	142	143
Lower + left	2	156 ± 0.5	155	156
Upper + left	2	146 ± 1.0	145	147
Not according to EN 15204	2	153 ± 1.5	151	154
Not given	1	148		

Table 8: Total cell numbers of the centric diatom *Thalassiosira baltica* in 25 video clips of the counting test. The results are presented separately for different methods for counting the edges of the counting grid.

Method	N	Mean ± SD	Min	Max
Lower + right	13	99 ± 3.1	63	103
Upper + right	2	98 ± 2.0	96	100
Lower + left	2	104 ± 0.5	103	104
Upper + left	2	122 ± 21.0	101	143
Not according to EN 15204	2	102 ± 0.5	101	102
Not given	1	103		

Table 9. Reference values calculated from the test material of the phytoplankton counting test. Robust mean value, from which the outliers were removed, was used as an assigned reference value (in bold). For the statistical treatment the different methods for counting the edges of the counting grid were standardised to correspond the lower and right edge counting method.

Assigned reference value	Cyanobacterium	Pennate diatom	Centric diatom
Median (all results)	47	154	101
Mean (all results)	47	153	101
Robust mean (no of outliers)	**47 (0)**	**153 (2)**	**100 (1)**
Robust mean (all results)	47	154	101
Expert value			
Lower + right edges	48	155	101
Upper + right edges	54	139	99
Lower + left edges	50	157	102
Upper + left edges	45	149	101

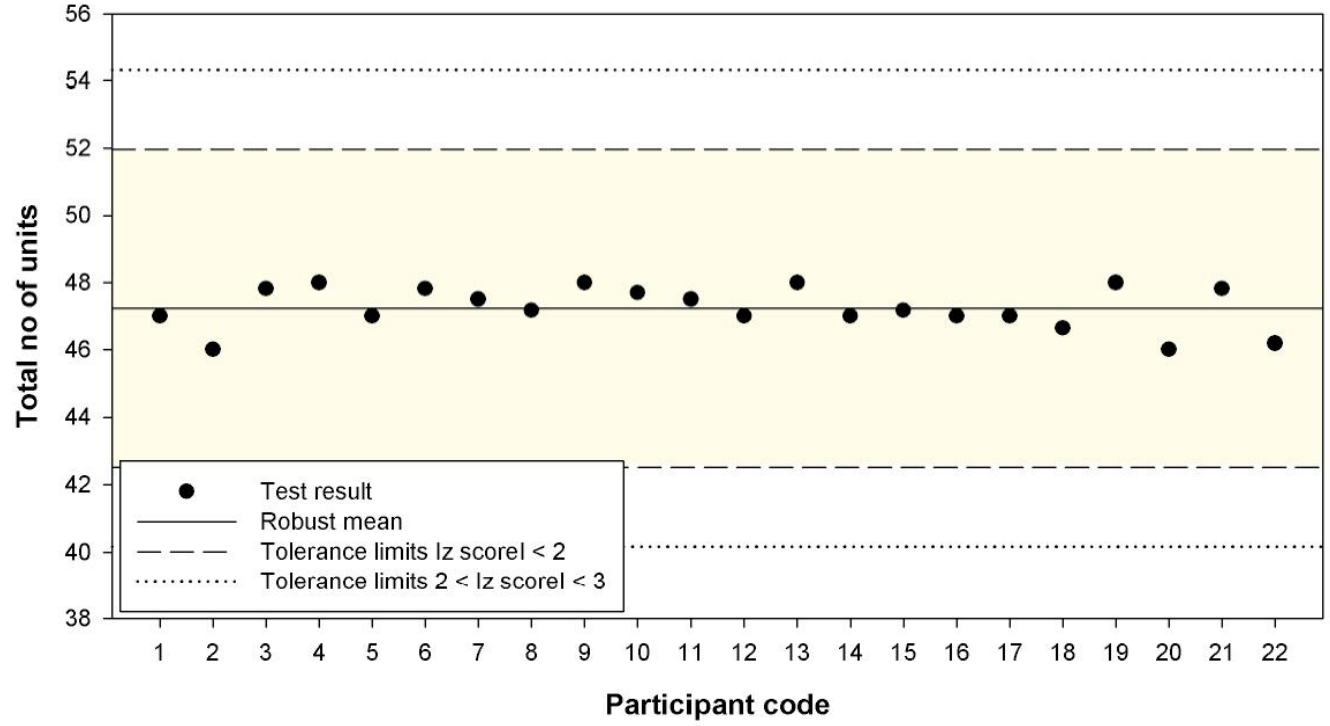

Figure 15. Standardised results of the counting test by participants for the filamentous cyanobacterium *Dolichospermum* (*Anabaena*) sp. $|z\ score| < 2$ = satisfactory (area in yellow), $2 \leq |z\ score| \leq 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

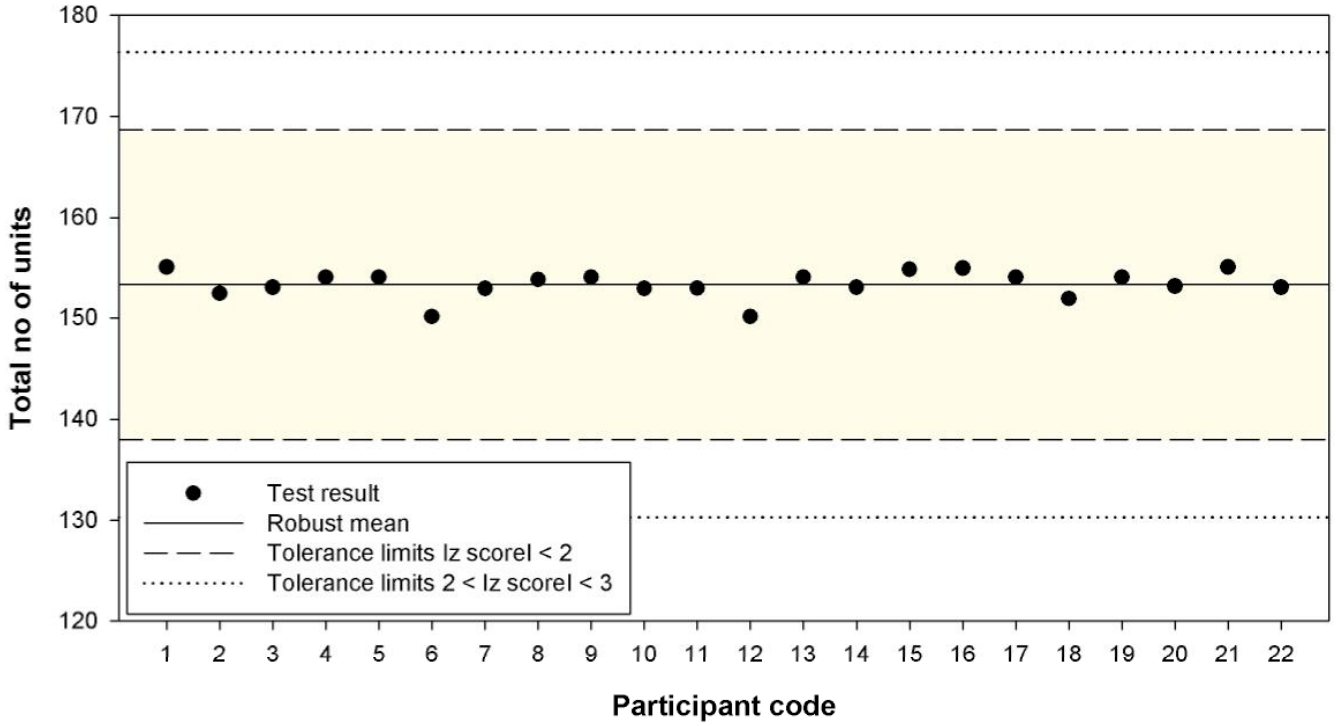

Figure 16. Standardised results of the counting test by participants for the pennate diatom *Diatoma tenuis*. $|z\ score| < 2$ = satisfactory (area in yellow), $2 \leq |z\ score| \leq 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

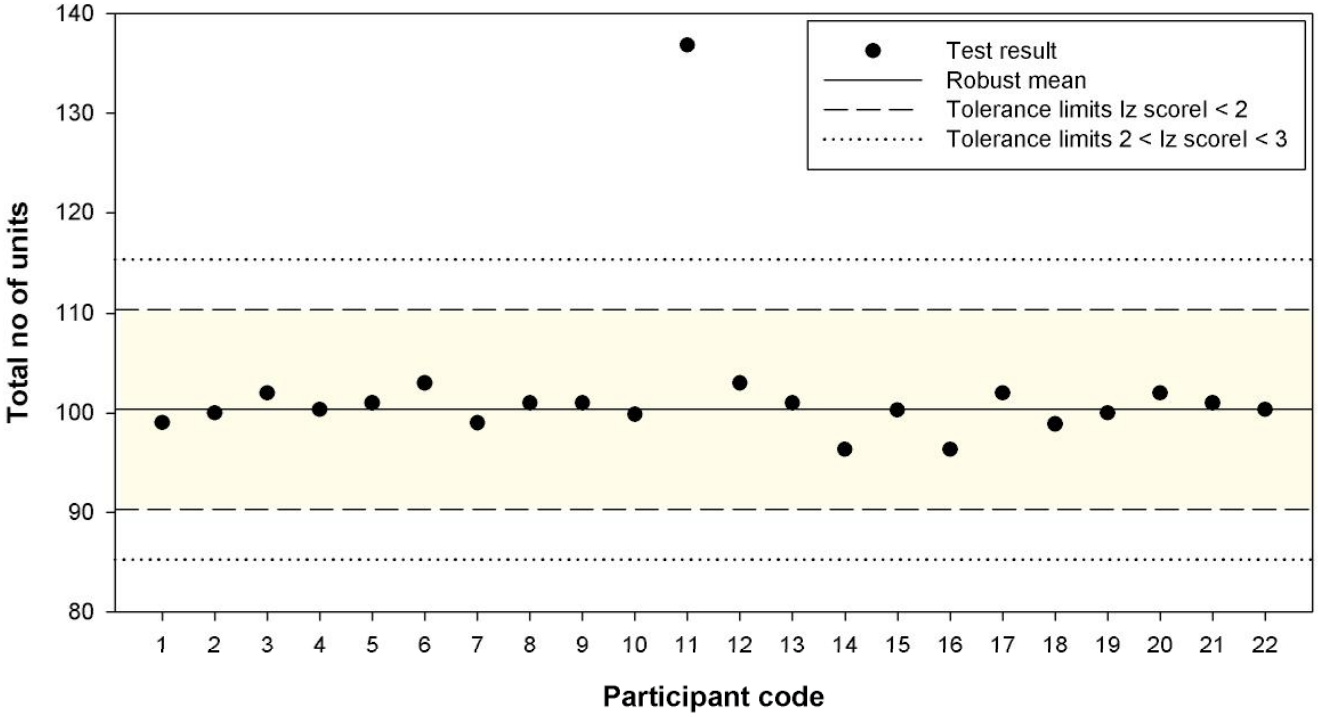

Figure 17. Standardised results of the participants of the counting test for the centric diatom *Thalassiosira baltica* from 25 video clips. $|z\ score| < 2$ = satisfactory (area in yellow), $2 \leq |z\ score| \leq 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

6.3 Measurement test

All 22 participants measured the requested cell dimensions. Most participants used a calibrated ocular micrometer in their measurements. Seven participants performed the measurements using an image analyser programme. Altogether 15 participants used phase contrast illumination, one participant used a relief phase contrast illumination, four participants used bright field illumination, and one of them either phase contrast or bright field illumination depending on the measured taxon. Differential interference contrast illumination and integrated light illumination were both used by one participant, respectively. Total magnifications used for the measurements ranged from 400x to 1000x. The ocular micrometer scales ranged from 1.0 µm to 3.0 µm.

In all, 17 participants performed all measurements satisfactorily, and five participants failed to correctly perform measurements of some dimension (Figs 18-20).

Table 10. Reference values and the count results of the expert panel for the measurement test. The robust mean where outliers were removed was used as an assigned reference value (in bold). Abbreviations: Dolicho = *Dolichospermum* sp., Phaeoda = *Phaeodactylum tricornutum* and Gymno = *Gymnodinium corollarium*, d = diameter, l = length and w = width (h = height).

Reference value	**Dolicho filament d**	**Phaeoda cell l**	**Phaeoda cell w**	**Hete tri cell h**	**Hete tri cell w (h)**
Median (all results)	6.4	22.0	2.9	19.4	17.59
Mean (all results)	6.4	22.0	2.9	19.6	17.4
Robust mean (no of outliers)	**6.4 (0)**	**22.1 (0)**	**2.8 (1)**	**19.4 (0)**	**17.5 (0)**
Robust mean (all results)	6.4	22.1	2.9	19.4	17.4
Expert value ± SD, n = 40	6.2 ± 0.4	22.4 ± 2.8	2.6 ± 0.6	19.6 ± 2.0	17.2 ± 1.7

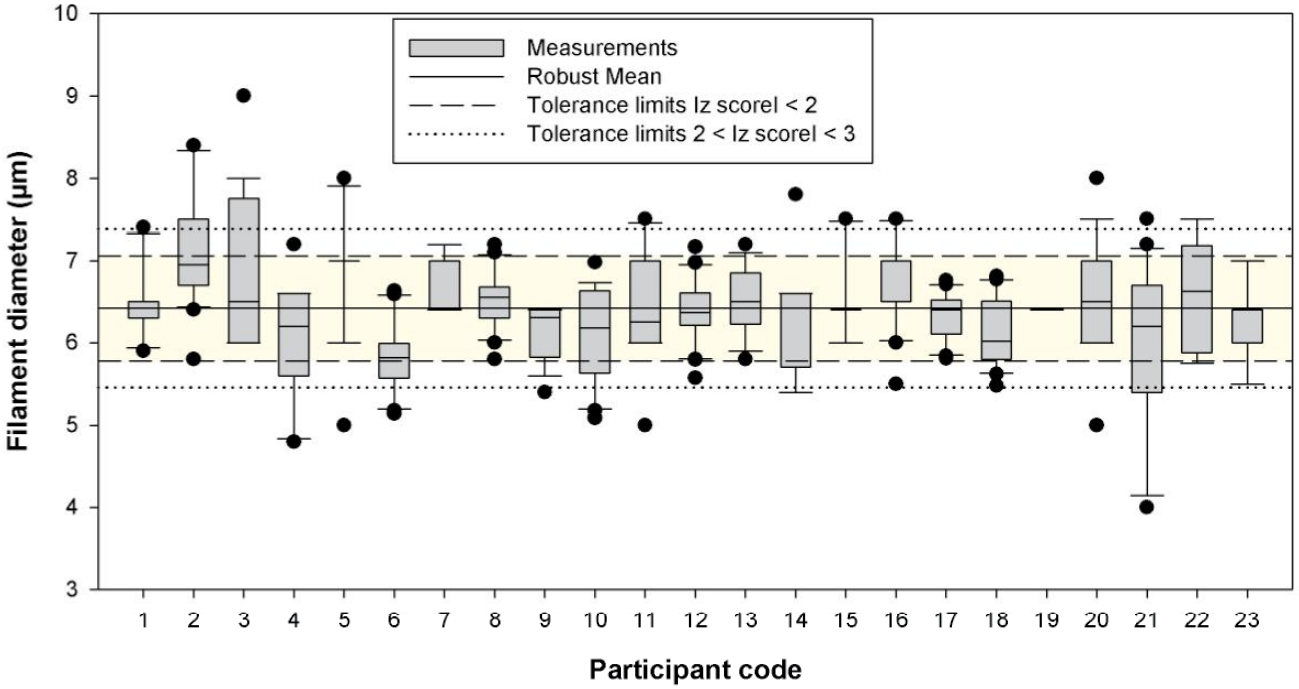

Figure 18. Box plot presentation (including median value, box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentiles and ●= outlying values) of the measurement results for the diameter of the cyanobacterium *Dolichospermum* (*Anabaena*) sp. Participant code 23 = expert reference measurements (n=40, two experts, measurements with ocular micrometer at magnifications 788x and 1000x with ocular micrometer scales of 1.6 and 1.8 µm, respectively). $|z\ score| < 2$ = satisfactory (area in yellow), $2 \leq |z\ score| \leq 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

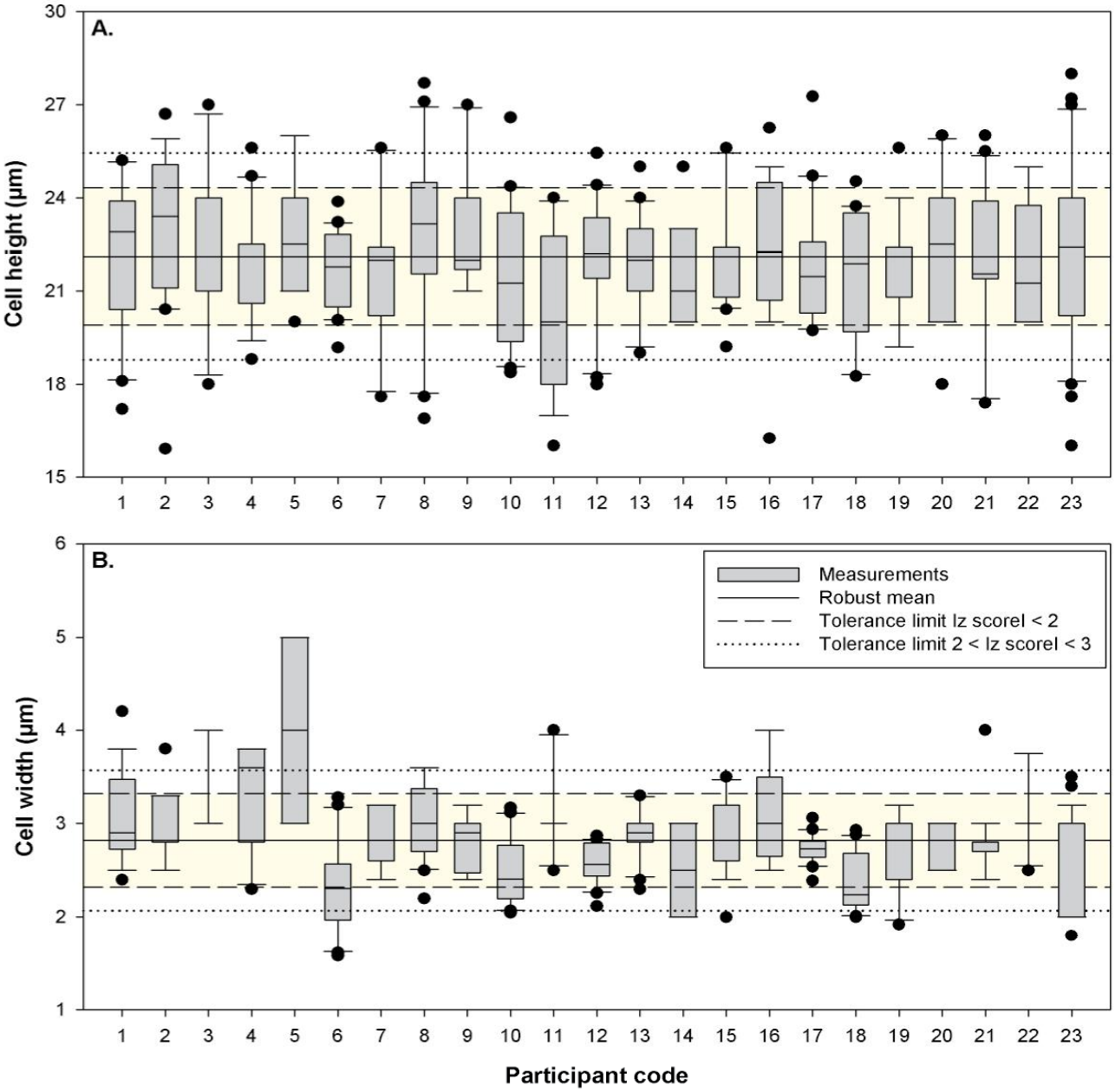

Figure 19. Box plot presentation (including median value, box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentiles and ●= outlying values) of the measurement results for the diameter of the pennate diatom *Phaeodactylum tricornutum*: A. height and B. width. Participant code 23 = expert reference measurements (n=40, two experts, measurements with ocular micrometer at magnifications 788x and 1000x with ocular micrometer scales of 1.6 and 1.8 µm, respectively). $|z\ score| < 2$ = satisfactory (area in yellow), $2 \leq |z\ score| \leq 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

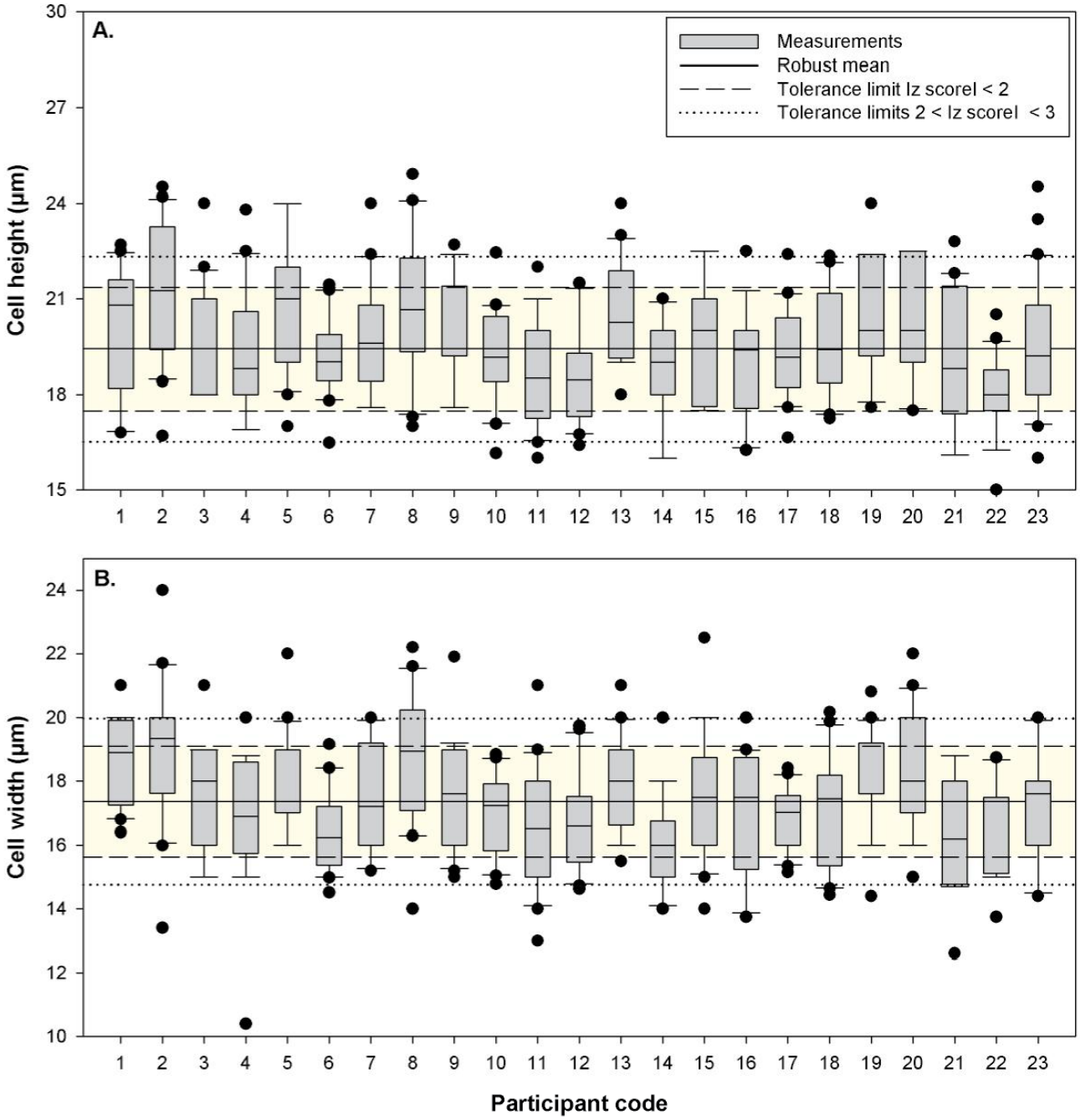

Figure 20. Box plot presentation (including median value, box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentiles and ●= outlying velues) of the measurement results of the diameter of the dinoflagellate *Gymnodinium corollarium*: A. height and B. width. Participant code 23 = expert reference measurements (n=40, two experts, measurements with ocular micrometer at magnifications 788x and 1000x with ocular micrometer scales of 1.6 and 1.8µm, respectively). $|z\ score| < 2$ = satisfactory (area in yellow), $2 \leq |z\ score| \leq 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

Due to high variation in the measurements of the cell width of the marine pennate diatom *Phaeodactylum tricornutum*, additional expert measurements were performed by the test organizers using both ocular micrometer and an image analyser computer programme. The width of the frustule was 2.6±0.5 µm (mean ± SD) when measured using ocular micrometer and 2.4±0.3 µm using image analyser programme which was a statistically significant difference (ANOVA $p = 0.001$, $n = 60$, $df = 1$). Therefore, the results of the participants no 6 and 18, who used image analyser programme and reported smaller mean widths that were originally regarded as questionable, were reconsidered as satisfactory.

In addition to measurements, participants were also asked to give the preferred shapes and equations for biovolume determinations (Table 10). This part of the test was not evaluated, nor included in the test diploma. This information was gathered to get an overview of the equations preferred by participants in the absence of a commonly accepted EU standard for the phytoplankton biovolume estimation.

Two geometric shapes and equations (square and rotational ellipsoid) were suggested for the cyanobacterium *Dolichospermum*. For the pennate diatom *Phaeodactylum tricornutum* and for the dinoflagellate *Gymnodinium corollarium* several different geometric shapes and equations were suggested. Half parallellipiped, prism on parallelogram base and rhomboid prism were considered as synonyms according to the draft proposal CEN TC230 WG2 TG3 (Phytoplankton biovolume determination). For *Phaeodactylum tricornutum* the proposed equations differed although the same geometric shape was reported, i.e. two different equations were suggested for a double cone and spindle.

The updated HELCOM PEG Biovolume Reporting (http://www.ices.dk/env/repfor/ index.htm), HELCOM (2008) and the draft proposal CEN TC230 WG2 TG3 suggests sphere ($V = \pi * d^3/6$) for *Dolichospermum* as the correct geometrical shape. For *Phaeodactylum tricornutum* the suggested geometrical shapes are: half parallelepiped ($V = l*w*h/2$; HELCOM PEG Biovolume Reporting) and half elliptic cylinder ($V = \pi * d^3/6$; CEN TC230 WG2 TG3 draft proposal). For the marine dinoflagellate *Gymnodinium corollarium* the suggested geometrical shape is sphere ($V = \pi * d^3/6$; HELCOM PEG Biovolume Reporting).

Table 10. Suggested geometric shapes and their equations for each taxon in the measurement component of the test. Abbreviations: V = volume, d = diameter, b = breadth, w = width, l = length.

Taxon	Geometric shape	Equation	n
Dolichospermum (cell diameter)	Sphere	$V= \pi*d^3/6$	20
	Rotational ellipsoid	$V= \pi*d^2*h/6$	1
	Roundish	Not given	1
Phaeodactylum tricornutum (cell dimensions)	Half parallellipiped (= prism on parallelogram base, rhomboid prism)	$V= l*w*h/2$	9
			3
	Double cone	$V= \pi*l*d^2/12$	1
	Double cone	$V= \pi*l*d^2/6$	2
	Spindle	$V= 2*\pi*b^2*l/15$	1
	Spindle	$V= 0.75*\pi*b*/6l$	1
	Rotational ellipsoid	$V= \pi*d^2*h/6$	2
	Cylinder	$V= \pi*d^2*h/4$	2
	Cuboid (box)	$V=l*h*w$ ($V=l*w^2$)	1
	Elongated	Not given	
Gymnodinium corollarium (cell dimensions)	Rotational ellipsoid	$V= \pi*d^2*h/6$	13
	Flattened ellipsoid	$V= \pi*h*d*0.75/6$	4
	Prolate spheroid	$V= \pi*d^3*0,9$	1
	Sphere-10%	$V= \pi*d^2*h/12$	1
	Triaxial ellipsoid	$V= \pi*h*(b^2*0.82/6)$	1
	Two cones	$V= \pi*d^2*h/12$	1
	Roundish	Not given	1

7. EVALUATION OF PERFORMANCE AND DISCUSSION

Phytoplankton are used for the assessment of ecological status of lakes and the Baltic Sea. Therefore, phytoplankton analyses require effective quality control procedures to assure the validity of phytoplankton results. A widely accepted way to monitor validity is to take part in proficiency testing schemes. The primary aim of the SYKE 8/2011 phytoplankton proficiency test was to help individual laboratories and institutes and their analysts to evaluate the reliability and comparability of their analyses and, if necessary, take remedial measures to improve the quality of their results. In the phytoplankton analysis the expertise of the analyst has a major importance. Therefore the test was carried out at an individual level, and the diploma also includes the name of the analyst who participated in the test.

Phytoplankton proficiency tests with natural samples typically include several sources of variation. The first source of variation may arise from the inhomogeneous material delivered to participants. Secondly, additional variation in test material may arise from the sample preparation, e.g. from an inadequate homogenization of samples and uneven settling. Virtual testing is an excellent method to minimise this and to produce as identical and homogenous material as possible, especially for the identification and counting tests.

Phytoplankton identification components of the SYKE 8/2011 tests proved more difficult than expected. Altogether 79% of the participants in the lake phytoplankton identification test reached the quality target of 75%. The corresponding percentage in the Baltic Sea phytoplankton identification test was lower, 67%. The most recent nomenclature for the taxa was not always used. This suggests that some participants were not aware or familiar with the most recent identification literature. The taxa that proved to be the most difficult to identify in the lake phytoplankton test were *Stephanodiscus binderanus, Kephyrion skujae* and *Cyanodictyon imperfectum* (*Cyanocatena imperfecta*). The species are all indicator species for eutrophy or oligotrophy in freshwaters.

In the Baltic Sea phytoplankton identification test, *Heterocapsa arctica* ssp. *frigida* Rintala & G. Hällfors proved to be the most difficult to identify. The taxon is a new subspecies, first described in Rintala *et al.* 2010, and therefore, some participants might have been unaware of the relatively recent update in the taxonomy of the species.

The success in the counting test was good and 95% of the participants performed all parts of the counting test satisfactorily. A detailed guidance on how to perform the counting test was not given, but participants were advised to follow the EN 15204 (2006) standard. This allowed the screening of the number of participants who followed the standard counting rules. The centric diatom, which also had dividing cells, caused a failure of one participant to perform this part of the counting test satisfactorily.

Altogether 90% of the participants performed all parts of the measurement test successfully. Preliminarily five participants failed to measure one of the five requested dimensions satisfactorily. The width of the pennate diatom *Phaeodactylum tricornutum* proved to be the most difficult to measure. Two participants measured the height of the diatom *Phaeodactylum tricornutum* unsatisfactorily, and the measurements of two participants were questionable. However, as mentioned before, after additional expert measurements, the results of the two participants, who used image analyser, were reconsidered satisfactory. Thus, only three participants failed to measure one of the five requested dimensions satisfactorily.

Errors in the measurements may arise e.g. from an incorrect calibration of the ocular micrometers or of the image analyser scale. Small cell size may also make the measurements difficult, especially if the ocular micrometer scale is not accurate enough for fine scale measurements. In the current test, *Phaeodactylum* cells were narrow and the size variation was relatively high, which made the measurements of the cell width difficult. Participants who used ocular micrometer and image analyser programme performed equally well in this part of the test.

The choice of cell shape and equation was asked because, in addition to measurements, the differences in the biovolume estimations may arise from the choice of the geometric shape. The high number of equations reported by participants emphasise the urgent need for a commonly accepted European standard for biovolume determinations.

The overall success in the phytoplankton proficiency test demonstrated excellent phytoplankton identification skills by a large number of participants. Majority of the participants was also able to perform phytoplankton counts and measurements satisfactorily. Only three participants performed one of the five requested measurements either questionably or unsatisfactorily. Two of the participants did not follow the EN 15204 standard but followed their own method in the counting test. Individual analysts benefit from participating in external quality assurance to maintain the quality and to further improve and harmonise the reliability of the phytoplankton analysis results.

The percentage (79%) of participants who reached the satisfactory quality target in the current lake phytoplankton identification test was similar to that (78% and 80%) of the other SYKE tests (SYKE 11/2006 and SYKE 7/2009, respectively; Vuorio et al. 2007a, 2010). In the Baltic Sea phytoplankton identification test the percentage of participants with a satisfactory performance (67%) was the same as in SYKE 7/2009 test, but lower than (90%) in SYKE 11/2006 test. The performance in the counting and measurement tests was excellent (95 and 91 %, respectively), although the measurement of the width of the diatom *Phaeodactylum* cell was difficult due to narrow cells.

8. COMMENTS SENT BY THE PARTICIPANTS

Only one participant commented the preliminary test results by the deadline of December 9, 2011. The comments concerned the phytoplankton identification: the freshwater taxa numbers 10, 14, 17 and the Baltic Sea taxa numbers 6, 12 and 17. The participant asked justification for the accepted identifications instead of the suggested identifications: 1) *Monoraphidium minutum* instead of *Raphidocelis subcapitata* (lake taxon no 10), 2) *Stauridium* (*Pediastrum*) *tetras* instead of *Pediastrum biradiatum* (lake taxon no 14), 3)*Tetraedron minimum* instead of*Teilingia excavata* (lake taxon no 17), 4) *Actinocyclus octonarius* instead of *Coscinodiscus granii* (Baltic Sea taxon no 6), 5) *Desmodesmus armatus* instead of *Desmodesmus communis* (Baltic Sea taxon no 12), and 6) *Chaetoceros constrictus* instead of *Chaetoceros holsaticus* (Baltic Sea taxon no 17).

The comments were forwarded to the external experts of the test who found no reason to change the scoring, as the requested test taxa were clearly identifiable and discernible from the ones proposed by the participant. The condensed comments by the external experts are as follows:

1) *Raphidocelis subcapitata* cells are narrowly cylindrical, horseshoe-shaped and equally thick throughout, sometimes thickened at one or both apices. The apices are widely rounded, often slightly inflated and obtuse (capitate). Cells are 1-3(-5) µm wide and (7-)10-20(-23) µm long, i.e. usually less than 10 times longer than broad enclosed within a mucilaginous envelope.The strongly bent cells of *Monoraphidium minutum* are as well horseshoe-shaped and slightly narrowing to rounded apices. Cells are 1-7 µm wide and 5-17 µm long, i.e. the cells are shorter relative to their width. *M. minutum* cells are not surrounded by mucilage.

2) A deep incision is characteristic for the inner cell of *Stauridium* (*Pediastrum*) *tetras*. The marginal cells are laterally united to apex and divided into two lobes by deep incisions. The cell wall is usually smooth. On the other hand, *Pediastrum biradiatum* typically has intercellular spaces and the inner cells are usually bilobed. A V-shaped sinus divides the marginal cells into two lobes which are dichotomously divided into two projections. The cell wall is slightly granulated or smooth.

3) The cells of *Tetraedron minimum* are flat and concave with four rounded corners terminating in a papilla-like cell wall thickening. Unlike *T. minimum*, the *Teilingia excavata* has two semi-cells which are broadly ovoid in face view and elliptical in side view. The four clearly visible attaching granules in the apex join the cells into short filaments. The *T. minimum* cell width of 4.5 µm was also smaller than the respective cell dimensions given in the literature for *T. excavata* (cell width 7-14 µm and length 7.5-14 µm).

4) In contrast to *A. octonarius,* the girdle view of *C. granii* is wedge shaped, even when focusing on the cell in valve view, while *A. octonarius* is cylindrical. The rosette might be difficult to observe in living cells (in contrast to diatom preparations), but it was observable in the video-clip. Besides, the areoli in *A. octonarius* are strictly radial, while in *C. granii* they are bent (according to Fourier mathematics) so that they resemble the order the seeds are in the sunflower head. *A. octonarius* is clearly iridescent in diatom preparations; in water mounts rainbow colors are not so clear. The size of the frustules varies very much in diatoms. During division the old valve always becomes the outer one and one halfalways becomes smaller than the other until the minimum size is reached, when usually sexual reproduction commences. In the low salinity of the Baltic Sea *C. granii* never reaches the sizes reported from ocean conditions.

5) The swollen ends which protrude slightly inside the setae are typical for *D. opoliensis*. The setae of *D. communis* are usually evenly bent, those of *D. opoliensis* slightly wavy and stronger.

6) Our specimen of*C. wighamii* was quite typical and fairly large. The windows are small, elliptical and what is most important, the setae emerge exactly from the comer of the cell when seen in side view. Also the apical setae are typical for *C. wighamii* running in the plane of the chain. On the other hand, in *C. holsaticus* the setae emerge well inside the edge of the cell, and as a result the cells are more strongly separated from each other than in *C. wighamii*, and the window is shorter but wider.

Overall, the comments obtained from participants were positive and in particular the rapid delivery of preliminary results was appreciated. One participant also commented that the reduction of the score by 0.5 points because of misspellings of the taxa was justifiable in the identification tests.

9. SUMMARY

The Finnish Environment Institute (SYKE) organized the third virtual proficiency test of SYKE based on filmed material. A total of 22 analysts from 20 organisations and 5 countries took part the test. The test material represented phytoplankton that typically occurs in freshwaters in the Northern Europe and in the Baltic Sea. The freshwater identification test concentrated on indicator species.

The test integrated three components: 1) phytoplankton species identification, 2) phytoplankton counting and 3) the measurement of cell dimensions. Both lake and the Baltic Sea phytoplankton identification tests consisted of 20 video-clips of 20 taxa. For the phytoplankton counting test 25 video-clips, representing 25 fields of view in a microscope, were filmed. In the measurement test dimensions of three selected taxa were asked to be measured from a Lugol´s solution preserved composite sample.

In the lake phytoplankton identification test altogether 79% of the participants reached the good quality target of 75%. The corresponding percentage in the Baltic Sea phytoplankton identification test was 67%. The success in the counting and measurement tests was excellent; 95% of the participants performed successfully in the counting test and the respective percentage in the measurement test was 91%. The majority of the participants demonstrated excellent phytoplankton identification skills and were also able to perform phytoplankton counts and measurements satisfactorily.

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PARTICIPANTS

Organisation	Country
GRONTMIJ (previously Carl Bro) Trine Warming Perlt	Denmark
Estonian Marine Institute (University of Tarto, Labcode 807) Annely Heinala	Estonia
Marine Systems Institute, Tallinn University of Technology Inga Lips	Estonia
Company Sanna Kankainen Sanna Kankainen	Finland
Ecomonitor Ltd Raino-Lars Albert	Finland
Marine Research Centre, Finnish Environment Institute (SYKE) Minna Ylä-Jarkko	Finland
Water and Environment Research of South-West Finland Sanna Autio Hanna Turkki	Finland
Pöyry Finland Ltd Eeva-Leena Anttila	Finland
Lammi biological station, University of Helsinki Jorma Keskitalo	Finland
Institute for Environmental Research, University of Jyväskylä Arja Palomäki	Finland
Essi Kangasaho	Finland
Ekoll Ab Susanne Gustafsson	Sweden
Erkenlaboratoriet Helena Enderskog	Sweden
Medins Biologi AB Jan-Erik Svensson	Sweden
Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU) Marlen Sunna	Sweden
Department of Systems Ecology, Stockholm University Helene Höglander	Sweden
Umeå Marina Forskningscentrum Chatarina Karlsson	Sweden
Landestalsperrenverwaltung des Freistaates Sachsen Helmut Kneschke (Untersuchunstelle Paulsdorf) Jan Trübsbach (Untersuchunstelle Plauen)	Germany
Leibniz Institute for Baltic Sea Research Susanne Busch Regina Hansen	Germany

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Author(s)	Kristiina Vuorio, Katarina Björklöf, Seija Hällfors, Reija Jokipii, Marko Järvinen, Maija Niemelä, Mirja Leivuori and Markku Ilmakunnas	
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Abstract	The Finnish Environment Institute (SYKE) organized in 2011 the third virtual phytoplankton proficiency test based on filmed material. A total of 22 analysts from 20 organizations and five countries took part the test. The test material represented lake phytoplankton indicator species of oligotrophy and eutrophy and phytoplankton that typically occurs in the Baltic Sea. The test included three components: 1) phytoplankton species identification test, 2) phytoplankton counting test and 3) phytoplankton measurement of cell dimensions. Material for the lake and Baltic Sea phytoplankton identification tests consisted of 20 taxa filmed on 20 video-clips. For the phytoplankton counting test 25 video-clips, representing 25 fields of view in a microscope, were filmed. In the measurement test the cell dimensions (diameter, height/width and length) of three selected taxa were asked to be measured from a Lugol preserved sample. In the lake phytoplankton identification test altogether 79% of the participants reached the good quality target of 75% of the maximum score. The corresponding percentage in the Baltic Sea phytoplankton identification test was 67%. The success in the counting test was excellent and 95% of the participants performed all three parts of the counting test successfully. Altogether 91% of the participants performed all three parts of the measurement test successfully. Majority of the participants demonstrated excellent phytoplankton identification skills and were also able to perform phytoplankton counts and measurements successfully. The results of the proficiency test highlighted to follow the EN 15204 guidance in the quantitative phytoplankton analysis and also emphasized the current need for a new standard for biovolume determinations.	
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Tiivistelmä	Suomen ympäristökeskus (SYKE) järjesti vuonna 2011 järjestyksessään kolmannen kasviplanktonin vertailukokeen, joka perustui virtuaaliseen materiaaliin. Yhteensä 22 kasviplanktonlaskijaa 20 eri organisaatiosta ja viidestä eri maasta osallistui testiin. Testimateriaali edusti makeitten vesien tyypillisiä ravinteisuuden ja ravinteisuuden indikaattorilajeja sekä tyypillistä Itämeren kasviplanktonia. Testi koostui kolmesta osiosta: 1) kasviplanktonin lajintunnistus, 2) kasviplanktonin laskenta ja 3) kasviplanktonin dimensioiden mittaus. Sekä järvi- että Itämeren kasviplanktonin tunnistusosiota varten kuvattiin 20 videota, joissa esiintyi 20 tunnistettavaa taksonia. Laskentatestiä varten kuvattiin 25 videota, jotka esittivät 25 näkymää mikroskoopissa. Soludimensioiden mittausta varten osallistujille toimitettiin Lugolin liuoksella säilötty näyte, josta tuli mitata kolmen eri taksonin solujen halkaisija, leveys/korkeus ja/tai pituus. Järvikasviplanktonin tunnistustestissä 79 % osallistujista saavutti tavoitetason 75 % maksimipistemäärästä. Vastaava prosenttiluku Itämeren lajintunnistusosiossa oli 67 %. Osallistujista 95 % suoritti kaikki kolme laskentatestin osiota hyväksyttävästi. Soludimensioiden mittaustestissä 91 % osallistujista menestyi hyväksyttävästi kaikkien kolmen taksonin mittauksissa. Suurin osa testiin osallistuneista suoriutui testin kaikista komponenteissa hyvin. Menestyminen laskentatestissä edellytti hyväksytyn EN 15204 standardin noudattamista. Eurooppalaisen biotilavuusstandardin puuttuessa mittausosiossa käsyttyjen solutilavuuksien määrittämiseen ehdotettujen geometristen kaavojen oikeellisuutta ei arvioitu.	
Asiasanat	vertailukoe, kasviplankton, järvet, Itämeri, lajintunnistus, laskenta, biotilavuuden mittaus	
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Sammandrag	Finlands Miljöcentral (SYKE) genomförde den tredje växtplankton provningsjämförelsen som baserade sig på virtuellt filmat material. Sammanlagt 22 experter från 20 organisationer och fem europeiska länder deltog i provningsjämförelsen. Testmaterialet bestod av indikatorarter i näringsfattiga och näringsrika sötvatten och typiska växtplanktonarter i Östersjön. Jämförelsen bestod av tre olika komponenter: 1) växtplankton identifieringstest, 2) växtplankton räkningstest och 3) mätningstest av cell dimensioner. För identifiering av sötvattenarter filmades 20 videotagningar med 20 olika sötvatten- eller brackvattentaxon. För räkningstestet filmades 25 videotagningar med 25 olika mikroskopvyer. För mätningstestet (cell diameter, -bredd och/eller höjd och längd) filmades ett Lugol inlagd prov som innehöll tre utvalda taxon. I identifieringstestet av sötvattentaxon nådde 79 % av deltagarna nivån för bra kvalitet, vilket var 75 % av den maximala poängsumman. Motsvarande siffra i identifieringstestet av brackvattentaxon var 67 %. Framgången i räkningstestet var bra och 95 % av deltagarna genomförde räkningstestet godtagbart. Sammanlagt 91 % av deltagarna genomförde mätningstestet godtagbart. Majoriteten av deltagarna visade utmärkta identifieringskunskaper och klarade sig utmärkt is alla tre komponenter av testet. Resultatet av provningsjämförelsen betonade betydelsen att följa EN 15204 standarden i räkningstestet. Bristen av en accepterad europeisk standard för bestämningen av biovolymer syntes i mängden av föreslag av geometriska former för de tre mätta taxonen.	
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